VOLUME 5 September 1956 NUMBER 5

THE AMERICAN JOURNAL OF

Tropical Medicine and Hygiene

Combining The American Journal of Tropical Medicine and The Journal of the National Malaria Society

OFFICIAL ORGAN OF
THE AMERICAN SOCIETY OF TROPICAL MEDICINE AND HYGIENE

PUBLISHED BIMONTHLY BY
THE WILLIAMS & WILKINS COMPANY
BALTIMORE 2, MARYLAND, U.S.A.

Copyright 1956, By The American Society of Tropical Medicine and Hygiene
Made in U.S.A.

THE AMERICAN JOURNAL OF

Tropical Medicine and Hygiene

Editor-in-Chief, L. W. HACKETT
Assistant Editor, FLORENCE M. FROST
School of Public Health, University of California
Berkeley 4, California

Editor, Tropical Medicine and Hygiene News
LOUIS J. OLIVIER, National Institutes of Health
Bethesda 14, Maryland

Editorial Board

HAMILTON H. ANDERSON
G. ROBERT COATNEY, *Chairman*
HAROLD FARNSWORTH GRAY
HERBERT G. JOHNSTONE
EDWIN H. LENNETTE
DONALD B. McMULLEN
HARRY MOST
LLOYD E. ROZEBOOM

OFFICERS OF THE
American Society of Tropical Medicine and Hygiene

President
ABA C. CHANDLER

President-Elect
JUSTIN M. ANDREWS

Vice-President
WILLARD H. WRIGHT

Secretary-Treasurer
JOHN E. LARSH, JR.
School of Public Health
University of North Carolina
Chapel Hill, North Carolina

Councilors:	*Term Expires*
CLAY G. HUFF	1956
SAMUEL W. SIMMONS	1956
ALBERT B. SABIN	1957
J. J. SAPERO	1957
MARSHALL C. BALFOUR	1958
THOMAS H. WELLER	1958
PAUL C. BEAVER	1959
WILLIAM C. REEVES	1959

Editor
L. W. HACKETT

Individuals interested in any phase of tropical medicine and hygiene, including all arthropod-borne diseases, medical care and human welfare are eligible for membership. Dues are $8 a year and include subscription to the American Journal of Tropical Medicine and Hygiene. An application blank is bound into this issue of the Journal for the use of interested individuals. Before being submitted to the Secretary, each application must be endorsed by two active members of the Society and a check for $8 (U.S. banks preferred) must accompany the application.

Entered as second-class matter March 16, 1921, at the Post Office at Baltimore, Maryland, under the act of March 3, 1879.
Subscription price $10.00 per volume. Single numbers, $2.00.

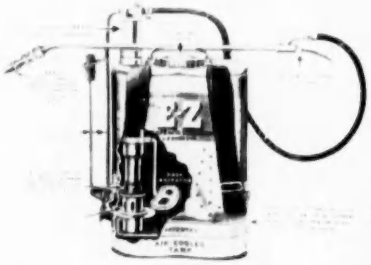
SMITH SPRAYERS

"For 70 Years Choice for Quality the World Over"

For Mosquito & Malaria Control

For Residual House Spraying or Larviciding Operations, Herculoy Silicon Bronze Sprayers are available in 4 gallon and smaller sizes and Armco Zinc-grip steel sprayers are also available in smaller sizes.

SMITH E-Z KNAPSACK SPRAYER



Maintains continuous high pressure, with only easy slow pumping. Pump entirely of brass to stand long hard usage. Tank solid copper or Armco zinc-grip steel. Equipped with best grade, high-pressure hose, 2-foot brass extension pipe with adjustable brass or fixed type nozzle. New shut-off control may be locked open for continuous spray. Capacity 4 to 5 gallons. Has form-fitting ventilated tank, keeping the back dry and comfortable. Carrying handle; and dasher type agitator which keeps liquid thoroughly mixed. Brass liquid strainer.

One in case. Shipping weight 40 lbs.

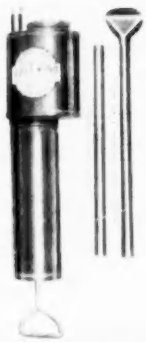
SEND FOR CATALOG

On our complete line of hand, continuous, compressed air, knapsack, bucket, wheelbarrow and barrel sprayers. Also hand and crank powder insecticide dusters. We are the originators of sprayers and our products are of outstanding quality in engineering, modern design and performance.

SMITH D.D.T. SPRAYER

The SMITH DDT Sprayer consists of a 4 gallon heavy welded galvanized corrugated steel tank, tested for hard service; equipped with brass air pump and brass air check valve; adjustable carrying strap with snap ends; air pressure gauge; 7-foot length of oil resisting hose; automatic shut-off control; 2-foot extension rod and all brass precision built adjustable or fixed type nozzles. A custom built, superior sprayer. Heavy duty industrial use.

One in case
Shipping weight 14 lbs.

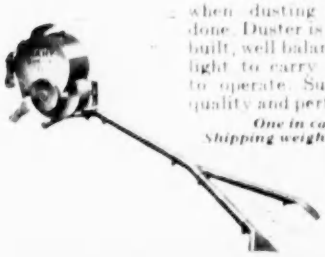


SMITH No. 16D DUST KING (quart-size) DUSTER

Unexcelled in performance. Has floating plunger, does not wear out, very easy to operate. Superior in design and construction. Approved by Experiment and Extension Services.

SMITH No. 44 GIANT DUSTER

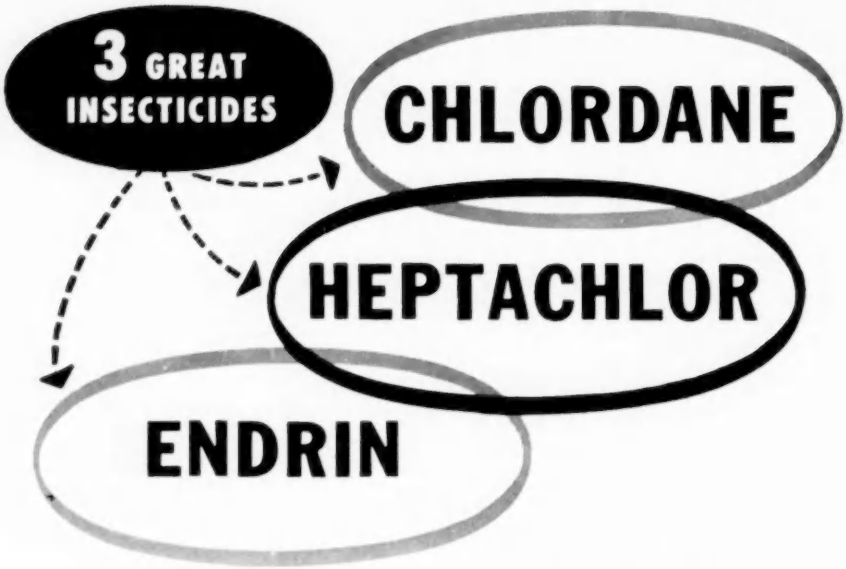
The Finest Made—Universally Approved



Unexcelled for mosquito and malaria control when dusting is being done. Duster is precision built, well balanced, very light to carry and easy to operate. Superior in quality and performance.

One in case
Shipping weight 12 lbs.

D. B. SMITH & COMPANY, 432 MAIN ST., UTICA 2, NEW YORK, U. S. A.
ESTABLISHED 1888



CONTROL THESE INSECTS

CHLORDANE: Ants, Armyworms, Blister Beetles, Boxelder Bug, Brown Dog Tick, Cabbage Maggot, Carpet Beetles, Cattle Lice, Chiggers, Cockroaches, Crickets, Cutworms, Darkling Beetles, Dog Mange, Earwigs, Fleas, Flies, Grasshoppers, Household Spiders, Japanese Beetle Larvae, Lawn Moths, Lygus Bugs, Mole Crickets, Mosquitoes, Onion Maggot, Onion Thrips, Plum Curculio, Sarcoptic Mange, Seed Corn Maggot, Sheep Ked, Silverfish, Sod Webworms, Southern Corn Rootworm, Strawberry Crown Borer, Strawberry Root Weevils, Sweet Clover Weevil, Tarnished Plant Bug, Termites, Ticks, Wasps, White Grubs, Wireworms...and many others.

HEPTACHLOR: Alfalfa Snout Beetle, Alfalfa Weevil, Ants, Argentine Ant, Armyworms, Asiatic Garden Beetle Larvae, Black Vine Weevil, Root Maggots, Clover Root Borer, Colorado Potato Beetle, Corn Rootworms, Cotton Boll Weevil, Cotton Fleahopper, Cotton Thrips, Crickets, Cutworms, Egyptian Alfalfa Weevil, European Chafer, Eye Gnats, False Wireworms, Flea Beetles, Garden Webworm, Grasshoppers, Japanese Beetle, Leaf Miners, Lygus Bugs, Mormon Cricket, Mosquitoes, Narcissus Bulb Fly, Onion Maggot, Onion Thrips, Rapid Plant Bug, Rice Leaf Miner, Salt Marsh Sand Fly, Seed Corn Maggot, Spittlebug, Strawberry Root Weevils, Strawberry Rootworms, Sugar Beet Root Maggot, Sweet Clover Weevil, Tarnished Plant Bug, Tuber Flea Beetle, Western Harvester Ant, White Fringed Beetles, White Grubs (June Beetles), Wireworms ...and many others.

ENDRIN: Budworms, Cabbage Worms, Cotton Boll Weevil, Cotton Bollworm, Cotton Fleahopper, Fall Armyworm, Grasshoppers, Hornworms, Leafworms, Rapid Plant Bug, Spiny Bollworm, Sugar Beet Webworm, Tarnished Plant Bug, Thrips.

WRITE FOR FULL PARTICULARS

VELSICOL CHEMICAL CORPORATION

VELSICOL

General Offices and Laboratories
330 East Grand Avenue, Chicago 11, Illinois

Foreign Division
350 Fifth Avenue, New York 1, N. Y.

REPRESENTATIVES IN PRINCIPAL CITIES

THE AMERICAN JOURNAL OF
TROPICAL MEDICINE AND HYGIENE

Vol. 5 *September 1956* *No.* 5

CONTENTS

Diagnosis of *Trypanosoma cruzi* infection by complement fixation. ELMER F. CHAFFEE, EARL H. EIFE, JR. AND JOHN F. KENT 763

Serological response of a rural Negro population to the Sabin-Feldman cytoplasm modifying test for toxoplasmosis. CALVIN L. GIBSON, DON E. EYLES, NEEL COLEMAN AND CLINTON S. SMITH 772

Final report on the first use of Puromycin for the treatment of sleeping sickness. CARLOS TRINCÃO, A. R. PINTO, ALMEIDA FRANCO AND ALFREDO NOGUEIRA 784

The incidence of *Trichomonas vaginalis* in the various age groups. LOUIS G. FEO 786

Poliomyelitis in Liberia. HENRY M. GELFAND AND MAX J. MILLER 791

Isolation of the virus of Ilhéus encephalitis from mosquitoes of the genus *Psorophora* captured in Honduras. ENDE DE RODANICHE 797

Transmission of Eastern equine encephalitis to horses by *Aedes sollicitans* mosquitoes. W. D. SUDIA, D. D. STAMM, R. W. CHAMBERLAIN AND R. E. KISSLING 802

Studies of dengue fever virus. (Hawaii mouse adapted.) in lactating hamsters. REGINALD L. REAGAN, FRANCES S. YANCEY AND A. L. BRUECKNER 809

Studies in shigellosis. VI. Observations on incidence and etiology of diarrheal disease in Egyptian adults. THOMAS M. FLOYD, JAMES W. BRAGG AND M. A. KADER 812

Biological and bacteriological studies on *Escherichia coli* in the housefly, *Musca domestica*. ROBERT L. INGRAM, JOSEPH R. LARSEN, JR. AND WARREN F. PITTEN 820

The relative egg producing capacity of *Schistosoma mansoni* and *Schistosoma japonicum*. DONALD V. MOORE AND JACK H. SANDGROUND 831

Intradermal reactions to *Schistosoma japonicum* and *S. mansoni* antigens in schistosome dermatitis cases. H. F. HSU AND D. J. AMEEL 841

Cytochemical studies on the hepatic tissue of mice following infections with *Schistosoma japonicum*. TOSHISADA SAWADA, KEIZO HARA, KOJI TAKAGI, YASUCHIKA NAGAZAWA AND SUKETAKA OKA 847

Pulmonary arteriovenous fistulas and arterial distribution of eggs of *Schistosoma mansoni*. J. LOPES DE FARIA 860

Thrombocytopenic purpura due to Fuadin (Stibophen). JULIO V. RIVERA, HECTOR F. RODRIGUEZ AND E. PÉREZ-SANTIAGO 863

Distribution of *Echinococcus* disease in northwestern Canada. R. W. WOLFGANG AND J. B. POOLE 869

Studies on *Echinococcus alveolaris* (Klemm, 1883) from St. Lawrence Island, Alaska. III. The histopathology caused by the infection of *E. alveolaris* in white mice. SAROJAM K. MANKAU 872

Mass treatment of ascariasis with a single dose of piperazine citrate. E. O. ATCHLEY, D. N. WYSHAM AND E. C. HEMPHILL 881

An evaluation of the standardized direct smear for egg counting in parasitological work. JOSÉ F. MALHOSANO 888

Murine typhus fever and rat ectoparasites in Puerto Rico. IRVING FOX 893

Some observations on the bionomics of the common mosquitoes of the Nile delta. HERBERT S. HERBERT AND BERNARD WEITZ 901

The influence of mating on egg production by *Aedes aegypti*. CALVIN A. LANG 909

An artificial feeding procedure for *Aedes aegypti* using sucrose as a stimulant. CALVIN A. LANG AND ROBERT C. WALLIS 915

Stream gradient as a criterion of lotic habitats suitable for *Australorbis glabratus* in Puerto Rico. HAROLD W. HARRY AND BILLY G. CUMBIE 921

Report of a case of human intestinal myiasis caused by *Leptocera scatophora*. DON W. MICKS AND J. WARREN MCKIBBEN 929

Errata 933

Books received 933

Book reviews. *Veterinary Parasitology*, by Lapage. *Annual Review of Entomology*, by Steinhaus *et al.* *Laboratory Identification of Pathogenic Fungi Simplified*, by Huzen *et al.* 933

THE AMERICAN JOURNAL OF

Tropical Medicine and Hygiene

THE AMERICAN JOURNAL OF TROPICAL MEDICINE AND HYGIENE is issued bi-monthly, appearing in January, March, May, July, September, and November. One volume a year is issued.

Accepted articles for which immediate publication would seem important will be inserted in the next issue of the journal to go to press if the author is willing to pay the manufacturing cost. The insertion of such articles will not affect the publication of other manuscripts awaiting their turn, since the inserted articles will constitute additional pages to the volume without additional cost to the subscriber.

Manuscripts and Books for Review should be sent to Dr. L. W. Hackett, School of Public Health, University of California, Berkeley 4, California.

Correspondence concerning business matters, including advertising, should be addressed to The Williams & Wilkins Company, Mount Royal and Guilford Avenues, Baltimore 2, Maryland, U. S. A.

Subscription price. $10.00 per volume, United States; $10.50 Canada; $11.25 elsewhere.

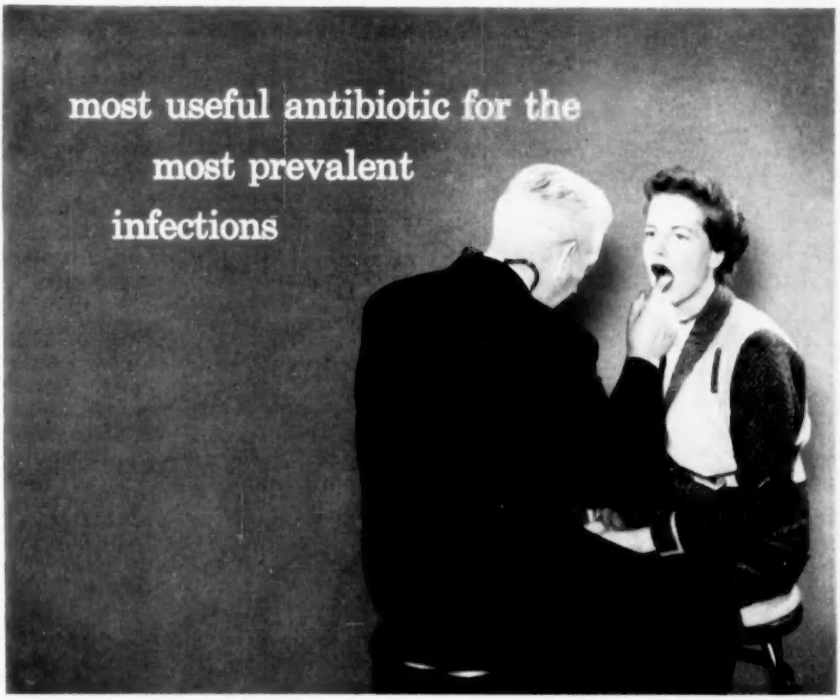
New subscriptions and renewals are entered to begin with the first issue of the current volume. Should any issue of the current volume be out of print at the time the subscription order is received, the pro-rata value of such numbers will be refunded to the subscriber.

Subscriptions should be renewed promptly. To avoid a break in your series, subscriptions should be renewed promptly. The publishers cannot guarantee to supply back issues on belated renewals.

Change of Address. Publisher must be notified 60 days in advance. Journals undeliverable because of incorrect address will be destroyed. Duplicates can be obtained (if available) from the publisher at the regular price of single issues.

THE WILLIAMS & WILKINS COMPANY
BALTIMORE 2, MARYLAND, U.S.A.

In writing to advertisers, please mention the journal—it helps.



'Ilotycin'

ERYTHROMYCIN, LILLY

Lilly

QUALITY / RESEARCH / INTEGRITY

Virtually all acute bacterial infections of the throat, nose, ear, and lung yield quickly. Yet, because the coliform bacilli are highly insensitive, the bacterial balance of the intestine is seldom disturbed.

'Ilotycin' kills susceptible pathogens of the respiratory tract. Therefore, the response is decisive and quick. Bacterial complications such as otitis media, chronic tonsillitis, and pyelitis are less likely to occur.

'Ilotycin' is notably safe and well tolerated. Staphylococcus enteritis and avitaminosis have not been encountered.

With usual dosages, gastro-intestinal hypermotility is not observed in bed patients and is seen in only a small percentage of ambulant patients.

Available as specially coated tablets, pediatric suspensions, I.V. and I.M. ampoules.

80TH ANNIVERSARY 1876 · 1956 / ELI LILLY AND COMPANY

NOTICE TO CONTRIBUTORS

The Journal welcomes papers which contribute to the purpose of the American Society of Tropical Medicine and Hygiene. This is the advancement of knowledge of tropical and subtropical medicine and hygiene through investigation, prevention, treatment and control of diseases and deficiencies. It draws upon medicine, biology, public health, nursing, engineering and allied fields of research and application.

The American Journal of Tropical Medicine and Hygiene combines the American Journal of Tropical Medicine and the Journal of the National Malaria Society and therefore has a special concern with the investigation, therapy and control of malaria in all parts of the world, as well as a broad interest in general parasitology and in arthropod borne diseases.

Papers are ordinarily published in the order of reception, which will entail a certain delay in their appearance because of the contractual limitation on the number of journal pages available in any year. Accepted papers which raise no questions of scientific priority may however secure earlier publication if the author or the institution sponsoring the paper will pay the publication cost, an estimate of which the Editor will be glad to obtain from the publishers. Such a subvention will be used to purchase extra pages, in excess of the regular page allocation for any issue of the Journal.

Authors are asked to cooperate by complying with certain standard practices in the preparation of their manuscripts in order to facilitate the editorial task:

Papers should be written in *English*.

Manuscripts should be typewritten using *double or triple spacing* on *one side* only of $8\frac{1}{2}$ x 11 inch paper, if available, and submitted in duplicate. Double spacing should be used throughout the manuscript *including the list of references*.

Tables should be typed on separate sheets and be serially numbered.

Figures and *photographs* should be enclosed separately and protected with cardboard to avoid creasing in the mails. Only glossy prints can be used. Line drawings and graphs should be submitted on white paper or tracing cloth, and not on cross section paper. Colored illustrations can only be reproduced at the expense of the author.

References should be arranged in alphabetic order according to authorship, with the date following the author's name, e.g.:

Snyder, T., and Meleney, H., 1941. The excystation of *Endamoeba histolytica* in bacteriologically sterile media. *Am. J. Trop. Med.* **21**: 63-73.

Citation in the text should be by author and date.

Proprietary names of drugs or chemicals registered by commercial pharmaceutical firms may not be used in the text if there is a generic name available in the U.S. Pharmacopoeia, New and Nonofficial Remedies of the A.M.A., or the latest editions of standard medical dictionaries. In certain cases where the generic name is relatively unknown, the registered name may follow it in parentheses in the title, at the first citation in the text, and in the conclusions.

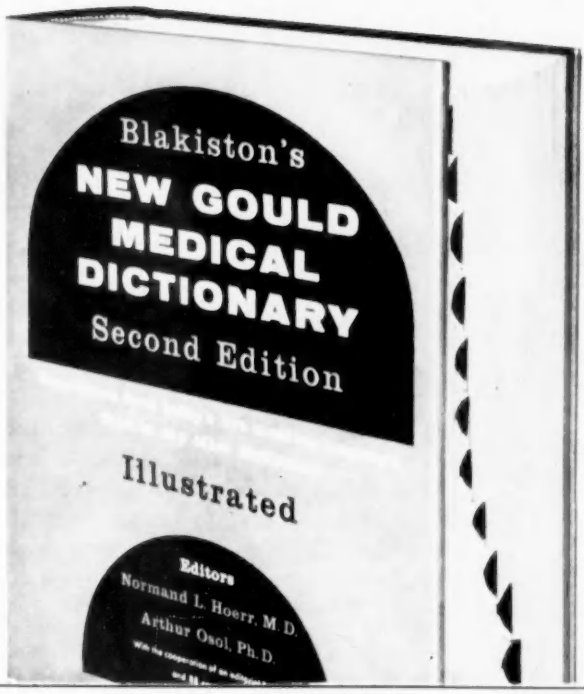
All papers should end with a brief summary.

Estimating costs of reprints. Where the cost of reprints must be known well in advance of publication in order to secure prior approval for their purchase, the Editor will be glad to furnish an estimate of the number of pages which the article will occupy in the Journal.

In writing to advertisers, please mention the journal—it helps.

Just off press with more terms of today's LIVE medical language than any other dictionary

1163 pages
252 illus. on 15 plates
129 in color
Standard Edition
Hard or Flexible cover
$11.50



Includes 12,000 new entries and 8,000 changes within definitions

Modernized spellings and usages throughout

New definitions on blood groups

Large accurate anatomical drawings

New Second Editon

Blakiston's New Gould Medical Dictionary

Edited by Normand L. Hoerr, M.D. and Arthur Osol, Ph.D.
with the cooperation of an editorial board and 88 contributors

Among the many features of the New Second Edition

• Defines new antiarthritic compounds. • Gives new and exact definitions from many specialists in cardiology, hematology, immunology, microbiology (with modernization of names of genera), neurology, poliomyelitis, radiation medicine, steroid hormones, syphilology, virus diseases, and aviation medicine. • Extends the coverage of biochemical terms. • Increases the coverage of terms from organic medical chemistry. • Adds a new table on isotopes and expands the table on hormones, on enzymes, and on vitamins.

Just Published

***Sawitz*—Medical Parasitology.** *New 2nd Edition*

By WILLIAM G. SAWITZ, M.D., Professor of Parasitology, Associate in Medicine, The Jefferson Medical College of Philadelphia. The short, concise, to the point style of the first edition is maintained in this new edition, which gives an understanding of the basic how's and why's of parasitic disease. It deals with the Protozoa, Helminthes, and Arthropoda which cause or transmit human disease. The chapter on treatment has been extensively revised. Better drawings, based on the teaching experience of the author, have been used to show the essential diagnostic points more clearly. A synopsis has been added to give an overall view of various phases of medical parasitology. Each parasite is discussed as to its life cycle, its effects on the human body, and diagnosis and prevention of the disease it causes or carries. 342 pages, 6 x 9, 90 illustrations (43 new). $6.00.

BLAKISTON DIVISION, McGRAW-HILL BOOK COMPANY, INC.
330 WEST 42nd STREET, NEW YORK 36, NEW YORK

The essential features of the virus and rickettsial diseases of man

SECOND EDITION

Textbook of VIROLOGY

FOR STUDENTS AND PRACTITIONERS OF MEDICINE

By **A. J. Rhodes, M.D., F.R.C.P.,** *Research Associate, Connaught Medical Research Laboratories, and Professor of Virus Infections, School of Hygiene, University of Toronto; Virologist, Hospital for Sick Children, Toronto; and* **C. E. van Rooyen, M.D., D.Sc., M.R.C.P.,** *Research Member, Connaught Medical Research Laboratories, and Professor of Virus Infections, School of Hygiene, University of Toronto*

Second edition rewritten from cover to cover, owing to the rapid advances in the field of virology. Nothing short of drastic rewriting would have sufficed to cope with the new developments.

More space given to the specific treatment of virus and rickettsial diseases as currently practiced in North America.

More attention paid to laboratory diagnostic procedures of proven and practical value.

Selected bibliographies for further reading appended to each chapter.

Many new illustrations.

CONTENTS

The Fundamental Characteristics of Virus Infections • Skin Diseases • Exanthemata • Respiratory Diseases • Venereal Diseases • Eye Diseases • Arthropod-borne and Tropical Fevers • Infectious Hepatitis and Serum Jaundice • The Coxsackie Viruses • Encephalomyocarditis and Encephalomyelitis of Animals • Neurotropic Virus Diseases • Rickettsial Infections

568 pp., 76 figs., $8.00

THE WILLIAMS & WILKINS COMPANY

Mt. Royal and Guilford Aves. Baltimore 2, Maryland

In writing to advertisers, please mention the journal—it helps.

THE AMERICAN SOCIETY OF TROPICAL MEDICINE AND HYGIENE

APPLICATION FOR MEMBERSHIP

To the Membership Committee:

The following individual interested in tropical medicine and hygiene is proposed for active membership.

(TYPE OR PRINT)

Name: ..... (Last) ..... (First) ..... (Middle)

Mailing Address: Street ..... City .....

State ..... Country .....

Date of Birth ..... Place .....

Special Field of Interest .....

Educational Institution, Graduate and Postgraduate ..... Degree ..... Date .....

Attach to the application a list of professional positions held, membership in scientific societies, and a list of major publications.

*Proposed by

1. .....

2. ..... Signature of Applicant

*Sponsorship by two active members is obligatory.

Return this application to Dr. John L. Larsh, Jr., Secretary-Treasurer, School of Public Health, University of North Carolina, Chapel Hill, North Carolina, U. S. A.

(A check for $10.00 U. S. funds requested, must accompany this application.)

for the control of insects by SPRAYING

Proper atomization plus uniform distribution are the spray characteristics that make precision-built TEEJET nozzles ideal for the spraying of insecticides. Full range of interchangeable orifice tips provide capacities for every type of chemical and every method of application. Complete nozzle includes built-in strainer with monel metal screen.

SPRAYING SYSTEMS CO.

3234 Randolph Street • Bellwood, Illinois

SPRAYING SYSTEMS TeeJet SPRAY NOZZLES

• INTER-CHANGEABLE ORIFICE TIPS

TRIGGER TEEJET

Quality built for hand spraying. Valve body drop forged brass with stainless steel valve stem. Self seal packing. Valve seat replaceable. Trigger lock.

Any TeeJet tip fits the Trigger TeeJet . . . also curved and straight extensions.

• TEEJET NOZZLES BUILT IN MALE OR FEMALE CONN

. . . . . WRITE FOR CATALOG 30

In writing to advertisers, please mention the journal—it helps.

FIRST . . . AND BEST!

TODD INSECTICIDAL FOG APPLICATOR

TIFA

Successfully Used for over 11 Years in Community Fly and Mosquito Programs

Tifa was first in the field of fog applicators. Tests have proved it best in the field for space control around the community — on the dump, over pools, in suburbs, everywhere insects swarm! This amazing machine discharges newly-developed insecticides as well as the older types in a true, clean fog which spreads quickly and permeates the deepest crevices. This ability means a better insect control program for your community. Put Tifa to work for you!

EFFICIENCY — Uses insecticides in solutions, emulsions or suspensions.
Produces penetrating fog, dry or wet as you wish.
FEATURES — Droplet Control ranges from ½ to 200 Microns.

CARRYING UNDERWRITERS' LISTING SEAL

Write for Illustrated Folder with Full Details and Official Comments

PRODUCTS DIVISION
TODD SHIPYARDS CORPORATION

HEADQUARTERS:
Columbia & Halleck Streets, Brooklyn 31, N.Y.
PLANT:
Green's Bayou, Houston 15, Texas

Practical, thorough guide to . . .

THE ECZEMAS

A SYMPOSIUM BY TEN AUTHORS

Edited by L. J. A. Loewenthal, M.D.

CONTENTS INCLUDE:

Contact and allergic eczemas; atopic dermatitis
Histopathology of eczema
Cutaneous resistance to acids, alkalies, commercial solvents
Role of Bacteria in Eczema
Seborrheic dermatitis
Disseminated and endogenous eczemas
Eczematous eruptions with special features
Diagnosis and treatment: general and particular
Appendices on patch testing and formulae

267 pp. 77 figs. 10 tables Index $7.50

THE WILLIAMS & WILKINS COMPANY
Mt. Royal and Guilford Aves. Baltimore 2, Maryland

In writing to advertisers, please mention the journal — it helps.

Animal Agents and Vectors of Human Disease

By ERNEST CARROLL FAUST, M.A., Ph.D.

The William Vincent Professor of Tropical Diseases and Hygiene and Head, Division of Parasitology, Department of Tropical Medicine and Public Health, The Tulane University of Louisiana, New Orleans

PARASITISM constitutes one of the major phenomena of the living world, and therefore one of its most interesting and challenging subjects. A highly important phase of parasitology is the broad area involving *animal agents and vectors, and their relation to disease*. Dr. Faust, internationally known for his work in this field, presents a full discussion of those parasites which produce human disease. The etiology, epidemiology, pathogenesis, symptomatology, diagnosis, treatment, and control of this group of infections are considered in detail.

The presentation is intentionally not exhaustive, yet the volume is much more than an outline syllabus. In extensive, helpful tables in the introductory chapters, Dr. Faust tells where each parasite under discussion is found, its usual infective stage, portal of entry, primary and secondary sites, usual reservoir hosts (monkeys, dogs, rats, etc.), and other obligate hosts and vectors. Pathologic effects and clinical manifestations produced by animal agents of disease, how to diagnose them, the most satisfactory therapeutic procedures and methods of control, etc., are presented in a similar manner. Subsequent chapters give detailed information on each agent or vector as it is considered individually.

Morphologic features of the organisms involved and the clinical aspects of the diseases which they produce, are elucidated by numerous helpful illustrations, many of which were made for this book. Ample references conclude each chapter. This important and timely work is designed for use by zoologists, epidemiologists, pathologists, practicing physicians, laboratory diagnosticians and public health workers.

660 Pages. 216 Illustrations and 9 Plates, 1 in Color. 12 Tables. $9.75.

Published January, 1955

LEA & FEBIGER WASHINGTON SQUARE PHILADELPHIA 6 PA.

notes from a
MICROSCOPIST'S
NOTEBOOK

FUCHSIN BASIC-SPECIAL
CERTIFIED
for Flagella Staining

This recent addition to our line of Commission Certified Stains provides an important new diagnostic aid to the medical profession. Its production and certification was undertaken by National Pharmaceutical Laboratories as one more step in providing an extensive line of biological stains, indicators and reagents for the microscopist.

For your convenience in ordering, this stain is catalogued:

#719 FUCHSIN BASIC—SPECIAL (for Flagella Staining)
10 gms. $1.00 25 gms. $2.00 50 gms. $3.70 100 gms. $7.00
200 gms. $12.65
plus 50¢ certification label charge

NATIONAL BIOLOGICAL STAINS and INDICATORS
PRODUCTS OF THE PHARMACEUTICAL LABORATORIES
NATIONAL ANILINE DIVISION
ALLIED CHEMICAL & DYE CORPORATION
40 RECTOR STREET, NEW YORK 6, N. Y.

NATIONAL PHARMACEUTICALS
Allied Chemical

DIAGNOSIS OF *TRYPANOSOMA CRUZI* INFECTION BY COMPLEMENT FIXATION

ELMER F. CHAFFEE, EARL B. FIFE, Jr.,[1] AND JOHN F. KENT

Department of Serology, Walter Reed Army Institute of Research, Walter Reed Army Medical Center, Washington, D. C.

The diagnosis of American trypanosomiasis (Chagas' disease) presents special problems, particularly in the chronic stage when the trypanosomes appear in the blood only intermittently and in small numbers. Under the circumstances, xenodiagnosis may afford the only means of demonstrating the parasite. The procedure is laborious, however, and requires special facilities for rearing triatomes free of *Trypanosoma cruzi*. As a result considerable attention has been given to practical serodiagnostic methods. The possibility of such a solution to the problem was first considered by Guerreiro and Machado (1913). On the basis that *T. cruzi* occurs in larger numbers in the host tissues than in the blood they prepared an aqueous extract of spleens from heavily-infected dogs; with this antigen they were able to demonstrate complement-fixing antibody in sera from 68 infected persons. However, nonspecific reactions were observed frequently in persons with syphilis, and even in healthy individuals. Liem and van Thiel (1941) have reviewed the many attempts to resolve this problem; while some improvement in specificity followed the introduction of cultured *T. cruzi* as the source of antigen (Kelser, 1936) nonspecific reactions continued to be encountered (Liem and Van Thiel, 1941). The extraction of specific trypanosomal components in organic solvents was reported by Romaña and Dias (1942) but later investigators (Muñiz and Freitas, 1944) found these antigens less satisfactory than the aqueous extracts.

The studies reported herein were suggested by the need for a more specific trypanosomal antigen. The methods used were similar to those applied in the purification of schistosomal antigen (Chaffee *et al.*, 1954). Preliminary extraction of the trypanosomes with anhydrous ether markedly reduced the frequency of nonspecific reactions, and specifically-reactive components proved readily soluble in alkaline buffer. The antigen was standardized for use with a precise complement fixation procedure. The present report gives a detailed account of the methods and the results obtained in serologic tests. The latter assume special interest in view of the apparent spread of indigenous Chagas' disease on the North American continent (Woody and Woody, 1955).

MATERIALS AND METHODS

Trypanosoma cruzi. The strain[2] used was one isolated in Brazil (1942) from a case of Chagas' disease. Stock cultures were maintained in 16 x 150 mm. Pyrex

[1] Present address: Fifth Army Medical Laboratory, St. Louis 2, Missouri.

[2] *Trypanosoma cruzi*, Brazil strain, obtained from the Laboratory of Tropical Diseases, National Microbiological Institute, National Institutes of Health, Bethesda, Maryland.

tubes containing blood agar slants (Senekjie, 1943) overlaid with a modified Locke's solution (von Brand *et al.*, 1946); they were incubated at 23° C., transfers to fresh medium being made every 10–12 days. Mass cultures for antigen production were grown in 500 ml. Erlenmeyer flasks containing 45 ml. of the blood agar overlaid with 50 ml. of Locke's solution. These were seeded with 0.5 ml. of overlay from stock cultures exhibiting satisfactory growth (40–50 organisms high dry field), and incubated for 12–14 days under standard conditions (23° C.). At the end of this period they were examined for possible contaminants, and the pure cultures collected in a sterile 4-liter flask by decanting the overlays carefully through a funnel lined with 6 layers of sterilized gauze. Trypanosomes remaining in the culture flasks were recovered by washing the agar surfaces three times each with about 5 ml. of sterile 0.85 per cent NaCl solution; the washings were passed through the gauze filter and combined with the bulk of culture.

To concentrate the trypanosomes the pool was distributed in 100 ml. Pyrex centrifuge tubes, and centrifuged for 30 minutes in the cold (5° C.) at 1,000 G; the supernates were discarded by careful aspiration through a Richards filter pump. The sediments were then suspended, each in about 5 ml. of sterile 0.85 per cent NaCl solution, and pooled in one of the 100 ml. centrifuge tubes. The other tubes were washed individually with 2–3 ml. of salt solution and the washings added to the pool. The material was then recentrifuged and the supernate discarded by aspirating carefully through the filter pump. The concentrated trypanosomes were suspended in about 5 ml. of salt solution and transferred by pipette to a 15 ml., tapered-bottom Pyrex centrifuge tube. Organisms adhering to the sides of the 100 ml. tube were recovered by washing twice with 2 ml. portions of salt solution, and the washings added to the 15 ml. centrifuge tube. The suspension was then freed of agar particles and debris by spinning for 2 minutes at 20 G in the refrigerated centrifuge. The supernate, which contained the organisms, was transferred by pipette to a 100 ml. Pyrex centrifuge tube and refrigerated at 3°–6° C. Trypanosomes entrained in the sediment were recovered by resuspending the material in 10 ml. of salt solution and spinning for 2 minutes at 20 G; the supernates from two such washings were combined with the bulk of harvest. The trypanosomes were then washed by bringing the volume of harvest to about 80 ml. with chilled salt solution and spinning for 30 minutes in the cold at 1,000 G; the supernate was discarded by careful aspiration through the filter pump. The washing process was repeated once using 80 ml. of salt solution, and the sedimented organisms transferred with a minimum of salt solution to a 10 ml. Pyrex ampoule. They were quickly shell-frozen (−78° C.) in a mixture of solid carbon dioxide and methyl cellosolve[1], then dehydrated under reduced pressure from the frozen state. After 16–18 hours' desiccation, the ampoule was sealed hermetically under vacuum, using a cross-fire torch, and stored in a unit refrigerated with solid carbon dioxide (−55° to −45° C.).

The cultured trypanosomes used in the early experiments were provided by William G. Johnes and John Saglett of the Department of Medical Zoology, Walter Reed Army Institute of Research, whose essential contribution is gratefully acknowledged.

[1] Ethylene glycol monomethyl ether, Union Carbide and Carbon Co., New York.

Antigen extraction. Glassware used in the preparation and standardization of antigen, and in the serologic tests, was cleaned scrupulously with sulfuric-chromic acid mixture (Hodgman *et al.*, 1950).

The antigen finally adopted was an alkaline buffer[1] extract of dried trypanosomes subjected to two preliminary extractions with anhydrous ether[2]. A glass tissue grinder powered with a variable-speed electric motor was used to facilitate extraction. Prior to ether extraction, both grinder and ether were chilled to −20° C. in a refrigerator. A 100 mgm. portion of the desiccated trypanosomes was then transferred quantitatively to the grinder cylinder, two ml. of cold anhydrous ether were added, and the mixture ground with the cylinder immersed in a calcium chloride, ice-water bath (−15° to −18° C.); the motor was run at low speed to minimize evolution of heat in the grinder. As soon as all solid material was in suspension an additional 6 ml. of cold ether were added, and grinding continued for 10 minutes. The ethereal suspension was poured into a chilled glass centrifuge tube and stored temporarily at 3° 6° C. Two ml. of cold ether were then added to the small residue in the grinder and grinding resumed for 2 minutes. This second suspension was combined with the first, and the whole centrifuged for 30 minutes in the cold at 1,000 G. The supernatant ether was decanted immediately thereafter, and the residue returned quantitatively to the grinder using portions of cold ether totalling 10 ml. This process of ether extraction was repeated once as described. After decanting the second ethereal extract, traces of ether vapor were removed from centrifuge tube and grinder by aspiration through the filter pump.

For the final extraction of antigen the trypanosome residue was returned quantitatively to the grinder using 8 ml. of alkaline buffer[1]. The mixture was then ground slowly for 10 minutes at room temperature, and the resulting suspension poured into a 20 ml. Pyrex centrifuge tube. The small residue in the grinder was recovered by grinding for two minutes with 2 ml. of buffer, and combined with the bulk of suspension. Four hours at 3° 6° C. were allowed for extraction. The suspension was then centrifuged for 30 minutes in the cold at 1,000 G and the supernate, representing the finished antigen, transferred carefully by pipette to a small beaker. One ml. volumes were dispensed in 10 ml. Pyrex ampoules, after which the contents were shell-frozen quickly in solid carbon dioxide and methyl cellosolve, then dehydrated under reduced pressure from the frozen state. After 16–18 hours desiccation the ampoules were sealed hermetically under vacuum, and stored at −55° to −45° C. When required for tests the contents of an ampoule were rehydrated with 1 ml. of distilled water. Antigen prepared and refrigerated in this manner remained stable in activity for periods of at least two years.

[1] The buffer consisted of 2.267 gm. 5,5 diethyl barbituric acid and 18.69 gm. Na 5,5 diethyl barbiturate in distilled water sufficient to make 1000 ml. The acid was dissolved in 500 ml. hot distilled water and added to 300 ml. of solution containing the sodium salt. The whole was cooled to room temperature and the volume adjusted to 1,000 ml. with distilled water; the pH of the solution was 8.6–8.7.

[2] Anhydrous Ether. Analytical Reagent Grade. Mallinckrodt Chemical Works. New York. Philadelphia. St. Louis.

TABLE 1

Effect of preliminary ether extraction upon the tendency of T. cruzi antigen to react with sera of healthy and syphilitic persons

Extraction*	Antigen	Healthy				Syphilitic				
		Number tested	Result†			Number tested	Result†			
			R	±	−		R	±	−	
A	0	95	1	3	91	21	3	6	12	
	I				95		2	6	13	
B	I	50		1	49	13	1	7	4	
	II				50			3	12	
C	II	120	2		118	13			13	

* The zero and roman numerals indicate the number of preliminary extractions with, subsequently, ether.

† All strongly reactive in serologic tests for syphilis.

‡ Classified as R (reaction), ± (+ or ± weak reaction), − (± or − no reaction).

For the comparative tests of specificity (Table 2), alkaline buffer extracts were prepared similarly from untreated trypanosomes and from organisms subjected to one preliminary extraction with ether, the antigens used in a given experiment were always from trypanosomes of the same lot.

Sera. Specimens for the studies were kindly provided by collaborating investigators in Brazil and Argentina. The sera representing South American trypanosomiasis were from well documented cases of *T. cruzi* infection, the diagnosis having been established either by demonstrating the organism in stained blood films, or by xenodiagnosis. Sera representing *Leishmania braziliensis* infection were obtained from native Brazilians, none of whom showed evidence of a current or past infection with *T. cruzi*; the leishmania in each instance had been discovered in typical lesions. Sera from both groups were preserved in tubes containing dry merthiolate[5] sufficient to give a final concentration of about 0.5 mgm. per ml., and were shipped by air mail to the Walter Reed Army Institute of Research.

The sera which served as controls were obtained from healthy donors among the laboratory staff and from military personnel undergoing physical examination at the Walter Reed Army Hospital. None of the persons concerned had resided in an area where trypanosomiasis is encountered, and all were negative in serologic tests for syphilis (Harris *et al.*, 1946; Kent *et al.*, 1948). The syphilitic sera were from patients accepted for chemotherapy at the Polk Health Center, Washington, D. C.; none of the persons selected had been exposed to trypanosomiasis, and all reacted serologically in the tests for syphilis. The sera were

[5] Sodium ethyl mercuric thiosalicylate, Eli Lilly and Co., Indianapolis.

stored at −20° C. Just prior to testing the required volume of thawed serum was heated for 30 minutes in the water bath at 56° C.

Complement fixation procedure. The methods used were a development of earlier quantitative procedures (Kent *et al.*, 1946). The reagent diluent was a veronal bicarbonate buffered salt solution (VBS) of pH 7.3–7.4 containing Mg^{++} and Ca^{++} in concentrations optimal for immune hemolysis (Mayer *et al.*, 1948). Preserved sheep's blood served as the source of erythrocytes (Bukantz *et al.*, 1946). Glycerolized hemolytic antiserum and desiccated guinea pig complement (C′) were obtained commercially. The standard volume for tests and titrations was 1.5 ml., 0.3 ml. aliquots being allotted the five reagents essential in complement fixation; adjustments to standard volume were made when necessary with VBS. The suspension of washed erythrocytes was adjusted spectrophotometrically to contain 5×10^8 cells per ml., and sensitized with an optimal concentration of hemolysin (Kent, 1947). The fifty per cent hemolytic unit (C'_{H50}) of complement was determined by a precise spectrophotometric technic. In the complement fixation test, 0.1 ml. of undiluted or diluted serum plus 0.2 ml. VBS was incubated 16–18 hours at 3°–6° C. with 0.3 ml. each of complement (5 C'_{H50}) and antigen. Sensitized erythrocytes (0.6 ml.) were then added and 30 minutes in the water bath at 37°C. allowed for hemolysis. The tests were read by visual comparison with standards simulating the possible findings; i.e., reaction (4+, 3+, 2+), weak reaction (1+, ±), and no reaction (−). Sera which reacted in qualitative tests were diluted serially in VBS, and retested quantitatively; titer was expressed as the reciprocal of the highest dilution yielding a reaction.

In standardizing the trypanosomal antigen, serial two-fold dilutions of reactive human serum were tested each with parallel dilutions of antigen; the serum was diluted in non-reactive serum from healthy individuals, the antigen in VBS. Results obtained in a representative titration appear in Table 1. In the given instance a 1:80 dilution of antigen was selected for diagnostic tests. It is note-

TABLE 2

Standardization of T. cruzi *antigen for the complement fixation test*

Antigen Diluted in Salt Solution 1:	Results* with *T. cruzi* Antiserum Diluted in Non-Reactive Serum 1:					Antigen Controls
	5	10	20	40	80	
10	4	2	±	—	—	—
20	4	3	1	—	—	—
40	4	3	1	—	—	—
80†	4	3	1	—	—	—
160	4	2	—	—	—	—
Serum controls	—	—	—	—	—	

* The recorded values represent the degree of reaction, the negative sign (−) no reaction.

† The dilution selected as optimal for diagnostic tests.

worthy that there was no evidence of anticomplementary activity in the antigen controls.

RESULTS

The efficacy of ether treatment in removing nonspecific components of the trypanosomes was determined in a series of experiments using sera from healthy and syphilitic individuals; the results are summarized in Table 2. In the initial experiment (A), seven of 95 normal sera reacted with antigen prepared from untreated trypanosomes; none reacted with an antigen derived from organisms once-extracted with ether. In the case of the syphilitic sera, however, one preliminary ether extraction had little effect upon the frequency of nonspecific reactions. Thus, eight of nine sera which reacted with antigen from untreated trypanosomes reacted also with the ether-extracted antigen. The effectiveness of further ether treatment was therefore investigated (Experiment B). Two ether extractions proved more effective than one, the number of reactions with syphilitic sera being reduced from 11 to 3; the reactions which persisted, moreover, were of low degree (wr). Fifty normal sera which were tested concurrently were negative in tests with the twice-purified antigen. Attempts to increase specificity further using three to five ether extractions were unsuccessful; there was evidence moreover that the additional treatments with ether caused some loss of specific reactivity. The experience with twice-ether-extracted organisms was therefore extended (Experiment C), the antigen being prepared from a third lot of trypanosomes. Sera from 15 syphilitic individuals proved negative in these tests, and only two of 150 healthy persons exhibited serologic reactions; the donors' histories in the latter instances revealed no evidence which could account for the findings.

The antigen prepared from twice-ether-extracted trypanosomes was then evaluated in tests of 96 persons, all well-documented cases of *T. cruzi* infection. Table 3 summarizes the findings. Ninety of the group reacted serologically, the serum titers ranging from 1 to 32 (mean, 14.5); only four sera showed weak reactions. In supplementary studies sera from persons infected with *L. brasili-*

TABLE 3

Results of the complement fixation test using T. cruzi *antigen in persons with American trypanosomiasis or* L. brasiliensis *infection*

Persons Tested		Persons Exhibiting the Given Serologic Result*		
Diagnostic status	Number	Reaction	Weak reaction	No reaction
T. cruzi infection	96	90† (94%)	4 (4%)	2 (2%)
L. brasiliensis infection	21	16‡ (76%)	3 (14%)	2 (10%)

* Classified as reaction (4+, 3+, 2+), weak reaction (1+, ±), or no reaction (−).

† The titers ranged from 1–32, the mean being 14.5.

‡ The titers ranged from 1–32, the mean being 14.1.

ensis were tested similarily to determine the frequency and degree of the cross reaction; the results are included in Table 3. Sixteen of the 21 individuals reacted serologically, the titers falling in the range, 1 to 32 (mean, 14.1); three sera yielded weak reactions.

DISCUSSION

Methods applied successfully in improving the specificity of schistosomal antigen (Chaffee *et al.*, 1954) proved effective also in removing nonspecific components of *T. cruzi*; preliminary extraction with ether considerably reduced the frequency of "false" serologic reactions. The effectiveness of the procedure suggests that cardiolipin (Pangborn, 1941) or similar lipids occur as normal components of the trypanosome. Their removal would account for the observed reduction in the number of reactions with syphilitic sera. It might also explain the decreased frequency of reactions with normal sera since the latter have been shown (Lund, 1942) to contain traces of antibody reactive with lipid antigens. It should be noted however that occasional unexplained reactions persisted even with the ether-purified antigen. The identification of the trypanosomal components responsible for these reactions seems a worthwhile subject for further investigation.

The capacity of the antigen to react with homologous antisera was not appreciably affected by ether treatment. Complement fixing antibody was demonstrable in 98 per cent of the individuals with proven *T. cruzi* infection (Table 3), and it is significant that only four of the sera yielded weak reactions. The serum titers, which ranged from 1 to 32 (mean, 14.5), were higher than those (1.7–4.6) recorded by Freitas and Almeida (1949). Some explanation for the improvement in sensitivity may lie in the fact that the trypanosomes were preserved immediately by desiccation from the frozen state, and extracted under conditions of time and temperature less likely to be associated with degenerative changes. In addition, a long period in the cold (16–18 hours at 3°–6° C.) was allowed for complement fixation, whereas Freitas and Almeida employed 4 hours at 3°–6° C., or alternatively 2 hours at 3°–6° C. plus ½ hour at 37° C. The relatively low sensitivity of their procedure may explain also the fact that "false" reactions were not observed with syphilitic sera.

Antibody reactive with trypanosomal antigen was encountered in persons with American leishmaniasis (Table 3), and occurred with approximately the same frequency as in the homologous infection. This observation was in agreement with that of Chang and Negherbon (1947), although the degree of the cross reaction in their studies is considerably lower. It contrasted with the findings of Pellegrino and Brener (1952), who encountered no serologic reactions with trypanosomal antigen in persons infected with *L. brasiliensis*. The occurrence of common antigens in the causative organisms is nevertheless consistent with their generic relationship. The divergence in experience must be attributed to differences in the methods of antigen extraction and in the complement fixation tests.

SUMMARY

An improved antigen for the complement fixation test was prepared by extracting desiccated, cultured *T. cruzi* with anhydrous ether in the cold (−15° to −18° C.) prior to final extraction with alkaline buffer. Preliminary extraction with ether markedly reduced the tendency of the antigen to react with sera from syphilitic and healthy individuals, and had no appreciable effect upon its capacity to react specifically with antibody produced in American trypanosomiasis (Chagas' disease).

Persons infected with *L. brasiliensis* could not be differentiated from individuals with American trypanosomiasis on the basis of their serologic reactions with *T. cruzi* antigen.

ACKNOWLEDGMENTS

Grateful appreciation is accorded our South American collaborators, whose generous contributions of sera made the study possible: they were Drs. José Almeida, Helvecio Brandão, José J. C. Filho, Oswaldo P. Forattini, José L. P. de Freitas, Abdon Hermeto, W. Lobato Paraense, Cecilio Romaña, and Mary B. Waddell. Special thanks go to Miss Donna Geib for soliciting and forwarding many of the specimens.

Appreciation is expressed also to Dr. Robert Stolar and his associates of the Polk Health Center, Washington, D. C., who provided all the syphilitic sera.

REFERENCES

VON BRAND, T., JOHNSON, E. M., AND REES, C. W., 1946. Observation on the respiration of *Trypanosoma cruzi* in culture, *J. Gen. Physiol.* **30:** 163–175.

BUKANTZ, S. C., REIN, C. R., AND KENT, J. F., 1946. Studies in complement fixation. II. Preservation of sheep's blood in citrate-dextrose mixtures (modified Alsever's solution) for use in the complement-fixation reaction, *J. Lab. & Clin. Med.* **31:** 394–399.

CHAFFEE, E. F., BAUMAN, P. B., AND SHAFILO, J. J., 1954. Diagnosis of schistosomiasis by complement fixation, *Am. J. Trop. Med. & Hyg.* **3:** 905–913.

CHANG, S. L., AND NEGHERBON, W. O., 1947. Studies on hemoflagellates. III. The specificity of serological reactions of *L. donovani*, *L. brasiliensis*, *L. tropica*, and *T. cruzi*, *J. Inf. Dis.* **81:** 209–227.

FREITAS, J. L. PEREIRA DE, AND ALMEIDA, J. O., 1949. Nova técnica de fixação de complemento para molestia de Chagas (reação quantitativa com antigeno gelificado de culturas de *Trypanosoma cruzi*), *O Hospital* (Rio de Janeiro) **35:** 787–800.

GUERREIRO, C., AND MACHADO, A., 1913. Da reação de Bordet e Gengou na molestia de Carlos Chagas como elemento diagnostico, *Brasil Med.* **24:** 225–226.

HARRIS, A., ROSENBERG, A. A., AND RIEDEL, L. M., 1946. A microflocculation test for syphilis using cardiolipin antigen. Preliminary report, *J. Ven. Dis. Inform.* **27:** 169–174.

HODGMAN, C. D., EDITOR, 1950. *Handbook of Chemistry and Physics*, 32nd edition, Cleveland: Chemical Rubber Publishing Co., pp. 2710.

KELSER, R. A., 1936. A complement-fixation test for Chagas' disease employing artificial culture antigen, *Am. J. Trop. Med.* **16:** 405–415.

KENT, J. F., 1947. A quantitative study of the complement hemolysin relation, *Science* **105:** 316–317.

KENT, J. F., BUKANTZ, S. C., AND REIN, C. R., 1946. Studies in complement fixation. I. Spectrophotometric titration of complement; construction of graphs for direct determination of the 50 per cent hemolytic unit, *J. Immunol.* **53:** 37–50.

KENT, J. F., BOYD, H. M., AND SANDERS, R. W., 1948. Cardiolipin antigen in the Kolmer complement-fixation test for syphilis, *Bull. U. S. Army Med. Dept.* **8:** 284–293.

LIEM, S. D., AND VAN THIEL, P. H., 1940–41. The complement-fixation test for Chagas' disease employing dried antigen, *Acta Leidensia* (Scholae Med. Tropica) **15–16:** 259–274.

LUND, H., 1942. The titration of traces of reagin, *Am. J. Syph. Gonor. & Ven. Dis.* **26:** 1–15.

MAYER, M. M., CROFT, C. C., AND GRAY, M. M., 1948. Kinetic studies in immune hemolysis. I. A method, *J. Exper. Med.* **88:** 427–444.

MUNIZ, J., AND FREITAS, G., 1944. Contribuição para o diagnóstico da doença de Chagas pelas reações de immunidade. I. Estudo comparative entre as reações de aglutinação e de fixação do complemento, *Mem. Inst. Oswaldo Cruz.* **41:** 303–333.

PANGBORN, M. C., 1941. A new serologically active phospholipid from beef heart, *Proc. Soc. Exper. Biol. & Med.* **48:** 484–486.

PELLEGRINO, J., AND BRENER, Z., 1952. A reação de fixação do complemento com antigeno de formas de cultura do *Schizotrypanum cruzi* na leishmaniose tegumentar americana, *O Hospital* (Rio de Janeiro) **42:** 971–980.

ROMAÑA, C., AND DIAS, E., 1942. Reação de fixação do complemento na doença de Chagas, com antigeno alcoólico de cultura do "Schizotrypanum cruzi", *Mem. Inst. Oswaldo Cruz* **37:** 1–10.

SENEKJIE, H. A., 1943. Biochemical reactions, cultural characteristics, and growth requirements of *T. cruzi*, *Am. J. Trop. Med.* **23:** 523–531.

WOODY, N. C., AND WOODY, H. B., 1955. American trypanosomiasis (Chagas' disease). First indigenous case in the United States, *J. A. M. A.* **159:** 676–677.

SEROLOGICAL RESPONSE OF A RURAL NEGRO POPULATION TO THE SABIN-FELDMAN CYTOPLASM-MODIFYING TEST FOR TOXOPLASMOSIS

COLVIN L. GIBSON, DON E. EYLES, NELL COLEMAN AND CLINTON S. SMITH

National Institutes of Health, National Institute of Allergy and Infectious Diseases, Laboratory of Tropical Diseases[1]

Since the introduction of the methylene-blue cytoplasm-modifying test ("dye test") as a quantitative measure of serum antibodies to *Toxoplasma gondii* (Sabin and Feldman, 1948), a number of investigators have recognized the value of this new tool in studying the epidemiology of human toxoplasmosis. Using this technic, several workers have investigated various segments of the population in different regions of the world in order to determine the prevalence and distribution of anti-toxoplasmic antibodies. Thalhammer (1953, 1955) and Kudicke and Pöhlig (1954) have recently reviewed the published reports of these studies.

As a result of these investigations, it is now recognized that antibodies to *Toxoplasma gondii* are much more widely distributed in the general population than the occurrence of clinical disease would suggest. Precise definition of the relationship between antibody prevalence and such factors as age, sex, race, and geographical distribution would contribute much to an understanding of the epidemiology of this disease and might suggest the mode of transmission, which at present remains totally unknown. One could hope that the various serological studies reported in the world literature would permit comparative analysis which would reveal some of the relationships involved. Unfortunately, however, such is not the case. For a number of reasons, the published reports do not lend themselves to easy comparison, and in respect to some factors they cannot be compared at all. Different investigators have used at least three distinct schemes for diluting the unknown sera so that reported titers frequently are not comparable; some workers have introduced variations in performing the test procedures which others do not use; at least two different definitions have been established for determining the end-point of the reaction; some authors have adopted the practice of reporting as "negative" all sera which fall below a certain "significant" titer, although there has been no general agreement as to what titer is considered significant; and finally, several of the published studies do not report the manner in which the test population was selected nor the distribution of positive titers with respect to age, sex, race, or titer level. These difficulties have been treated in some detail by Thalhammer (1953, 1955) and by Kudicke and Pöhlig (1954), to whose papers the reader is referred.

In order to obviate the difficulties enumerated above and to provide easily comparable data on the prevalence of anti-toxoplasmic antibodies with respect to age, sex, race, habitat, and other factors of possible epidemiological significance, the staff of this laboratory has undertaken a series of serological studies

[1] Located at the Medical School of the University of Tennessee, 874 Union Avenue, Memphis 3, Tennessee.

on various segments of the population in the area around Memphis, Tennessee. Precisely the same techniques are being used in all studies of this series, and all tests are being performed in the same laboratory. As a result, variations in technique can be excluded in considering any differences which may be detected between different population groups. The present report is the first in this series. So far as we are aware, it also is the first published study of the prevalence of anti-toxoplasmic antibodies in a Negro population group.

METHODS AND MATERIALS

In 1950, in connection with a mass venereal disease survey, we collected approximately four thousand sera from the rural Negro population of Fayette County, Tennessee, which is the first county to the east of the city of Memphis (Fig. 1). Collecting stations were set up at schools, churches and stores throughout the county, and sera were taken from all school children in the county as well as from all pre-school children and adults who voluntarily came to the collecting stations as a result of the pre-campaign publicity. It is thus possible that some selection in favor of health-conscious families is reflected in the sample of sera. The families represented in these collections are, almost without exception, tenant farmers concerned chiefly with the growing of cotton and corn. Most families own a cow, a few pigs, and several chickens. Dogs and cats are common throughout the county.

The present report is an analysis of 987 sera drawn from the large group described above. These sera were selected as follows: first, an equal number was selected at random from each one-year age group (ages 6–20) and from each ten-year age group (ages 21 and above); second, all available sera from persons below six years and above 71 years of age were selected to provide sufficient numbers for significant analysis in these groups; third, sera from all nine-year old children were included to permit comparison with similar urban age groups in later studies; fourth, sera were included from all persons living south of the Wolf River (see Fig. 1) because the intestinal parasites of these persons have been studied by members of our staff (Eyles *et al.*, 1953) and we intend in a future publication to explore possible correlations between dye test titers and intestinal

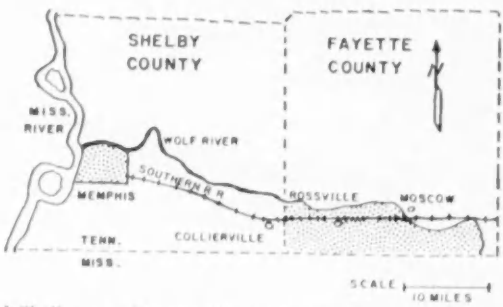


FIG. 1. Map of Shelby and Fayette Counties. The shaded region in Fayette County shows the area south of the Wolf River (see text).

TABLE 1

Number of sera tested, by age and sex

	Age in years						Total
	1-5	6-9	10-19	20-39	40-59	60+	
Males	27	186	197	34	32	27	503
Females	23	182	190	46	30	13	484
Total	50	368	387	80	62	40	987

parasites. About 60 per cent of the sera which we tested came from this latter area, in the southern third of Fayette County; approximately 30 per cent came from the middle third of the county; and about 10 per cent came from the northern third.

The 987 sera in our sample were tested by the original procedures stipulated by Sabin and Feldman (1948) and recently redescribed by Feldman (1954). All sera were tested undiluted, and at progressive four-fold dilutions (1:4, 1:16, 1:64, etc.) until the end point had been reached or exceeded by one dilution tube. Titers are reported on the basis of original serum dilutions. On numerous occasions we re-tested specimens to prove the reproducibility of our readings, and frequent comparative tests on unknown sera by our laboratory and several other research laboratories in the United States have always shown good agreement, thus lending confidence to our results. Table 1 shows the distribution, by age and sex, of the 987 sera which we tested. Although all age groups were represented in the study, it will be seen that about three-fourths of the sera came from children of school and teen age, with relatively few samples from the youngest and oldest age brackets.

RESULTS

Fayette County presents a relatively homogeneous topography. Only minor differences in elevation are found, but the county is traversed by two distinct river systems belonging to the Loosahatchie and the Wolf River watersheds. To determine whether there might be some difference in the distribution of toxoplasmosis related to these river valleys, we made a preliminary analysis of the data on a topographical basis, comparing persons living in the river bottoms with those living in the uplands. Since no consistent differences could be detected, the data for the entire county were thereafter considered as a unit.

Table 2 depicts the distribution of positive titers by age and sex. Within each age group there is close agreement between males and females in regard to the percentage of positive titers. The greatest divergence occurs in the 40–59 year age group, but neither here nor at any other age do our data show a significant difference between the sexes in their response to the dye test.

These data also show that, with the exception of the youngest age group, the percentage of positive titers in the population steadily increases with age. The reason for the discrepancy in the 1–5 year age group is not apparent. It may be

TABLE 2

Prevalence of positive sera (all titers) by age and sex (Data given in per cent)

	Age in years						Total
	1–5	6–9	10–19	20–39	40–59	60+	
Males	18.5	17.7	16.7	35.2	37.5	66.6	22.5
Females	17.4	14.9	17.9	32.6	46.7	61.5	21.0
Both sexes	18.0	16.3	17.3	33.8	41.9	65.0	21.8

a true difference, or it may be related to the fact that only fifty sera from the group were available for testing. It certainly is not due to passive transfer of antibodies from maternal blood, since the earliest age at which positive sera were found in the present series was three years, by which time all passively-transferred antibodies have long since disappeared.

The last line of Table 2 combines the data for the two sexes and illustrates more directly the increased prevalence of positive sera as the population becomes older. We find almost one hundred per cent increase between the 10–19 year old group, with 17.3 per cent, and the 20–39 year old group, with 33.8 per cent. Of course, the latter age grouping encompasses twenty years as opposed to the ten years included in the lower age group; but analysis of the data for the ages 20–29 (not shown in the table) reveals positive titers in 25.6 per cent of the sera tested. Thus there clearly is a marked increase of positive titers beginning at about age twenty.

At the same time there is a significant shift in modal titer, in accordance with the data shown in Table 3. Younger persons produce fewer positive sera, but they tend to have higher titers, whereas older persons are more frequently positive but tend to have lower titers. This situation is well illustrated in Figure 2, which shows the distribution of positive titers in the ages below twenty on the upper graph, and in the ages from twenty upward on the lower graph. In the younger group, the modal titer occurs at a dilution of 1:64 and a relatively large number of sera are positive at even higher dilutions. In the older age group, on the other

TABLE 3

Distribution of sera by age and titer (combined sexes)

Age	Number Tested	Number Neg.	Number Positive at Each Titer Level						
			Undil.	1:4	1:16	1:64	1:256	1:1024	1:4096+
1–5	50	41	0	0	2	2	4	1	0
6–9	368	308	6	1	9	20	13	8	3
10–19	387	320	14	4	16	16	9	6	2
20–39	80	53	3	3	10	4	5	0	2
40–59	62	36	5	6	8	5	0	1	1
60+	40	14	4	5	10	5	1	1	0
Totals	987	772	32	19	55	52	32	17	8

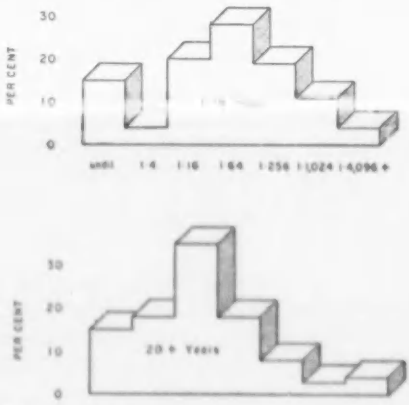


FIG. 2. Distribution of positive titers by age, showing shift in modal titer (both sexes combined).

hand, the modal titer occurs at a dilution of 1:16, with a strong tendency for positive sera to fall into the lower dilutions.

Since only one titer dilution separates the two modes, we thought it desirable to give statistical proof of the validity of this apparent shift in modal titer. A chi-square test on the data gave the values shown in Table 4. If there were no real shift of modal titer between the two age groups, then there should have been 67 low titer sera and 69 high titer sera in the younger age group, with 39 low titers and 40 high titers in the older age group. The observed number of sera in each category deviated widely from these expectations, with the younger age group showing a pronounced tendency to higher titers while the older age group produced a preponderance of low titers. The chi-square value derived from these data is 18.02. This gives a probability of 0.00004, indicating that the difference between the two groups is highly significant and that a very real shift of the modal titer occurs as the population ages.

Another interesting characteristic of the distribution pattern of negative and positive titers is illustrated in Figure 3, which shows the number of sera found at each titer level. (Note that the ordinate for the negative titers is not drawn to

TABLE 4

X^2 test to determine significance of shift in modal titer

Age (years)	Number Positive			
	Titer ≤ 1:16		Titer ≥ 1:64	
	Obs.	Exp.	Obs.	Exp.
1–19	52	67	84	69
20 or more	54	39	25	40

$X^2 = 18.02$ $P = 0.00004$

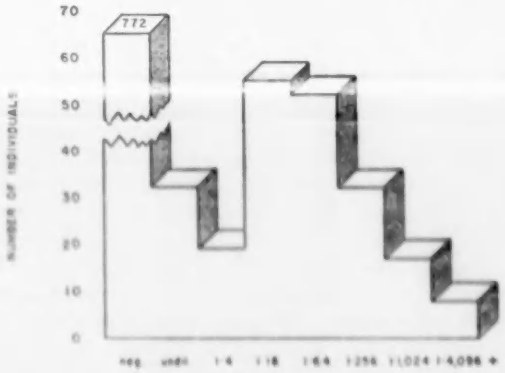


FIG. 3. Distribution of sera by titer showing bimodality (all ages, both sexes).

scale.) The curve shows a strong suggestion of bimodality, with maxima at or near the negative value and the 1:16 positive dilution, and a minimum at the 1:4 dilution. The large number of sera with titers positive at dilutions of 1:64 and higher serves to strengthen the bimodal nature of the curve and to emphasize the abnormally large number of undiluted positive sera. We do not know what significance, if any, this bimodal tendency has in the epidemiological pattern of toxoplasmosis.

Table 5 presents the prevalence of positive titers in the entire sample which we tested. The crude rates for each age group when all positive sera are counted are given in column 3 of the table. If we take into consideration the apparent bimodality of titer distribution previously discussed, and therefore exclude from our calculations those sera giving positive titers when undiluted or when diluted 1:4, then the crude prevalence rates given in column 4 will apply. Crude rates for all ages combined are given at the foot of columns 3 and 4.

Inasmuch as our sample was derived largely from the 6–20 year old age groups, we felt that it was desirable to standardize these "over-all" rates by relating them to the actual population of the county in which we worked. In this way we would compensate for the relatively small numbers of sera in the very young age groups and in ages above 20 years. The actual Negro population in 1950, the year in which our samples were taken, is given in Table 5, column 2. By applying the crude prevalence rates for each age group (as derived from our sample) to the actual population of each age group, we calculated the theoretical number of positive sera in the entire county. The total of these theoretical cases, when divided by the total population of 18,176 Negro persons registered in the census of 1950, gave standardized overall rates (both sexes, all ages) of 29.9 per cent for all positive titers, and 21.8 per cent for titers of 1:16 or higher. However, as Thalhammer (1955) has pointed out, these overall rates (even when standardized) are apt to be misleading in view of the tendency for positive titers to become more frequent with increasing age of the population.

TABLE 5

Crude rates for the prevalence of positive dye tests

Sex	Age (years)	Number tested (1)	Actual population (1950 census) (2)	Crude rates (per cent)	
				All positive titers (3)	Titers 1:16 or higher (4)
Males	1–5	27	1,576	18.5	18.5
	6–9	186	1,081	17.7	15.0
	10–19	197	2,193	16.7	12.8
	20–39	34	2,060	35.2	26.6
	40–59	32	1,614	37.5	21.9
	60+	27	898	66.6	44.3
Females	1–5	23	839*	17.4	17.4
	6–9	182	1,066	14.9	13.8
	10–19	190	2,147	17.9	12.7
	20–39	46	2,436	32.6	26.0
	40–59	30	1,568	46.7	26.7
	60+	13	698*	61.5	38.5
Both sexes	1–5	50	2,415*	18.0	18.0
	6–9	368	2,147	16.3	14.4
	10–19	387	4,340	17.3	13.9
	20–39	80	4,496	33.8	26.2
	40–59	62	3,182	41.9	24.1
	60+	40	1,596*	65.0	42.5
		987	18,176*		
Crude rates for all ages combined:	Males			22.5	17.2
	Females			21.0	16.2
	Both sexes			21.8	16.6

* Females less than three years of age and more than 84 years of age are not included, since our sample did not include any persons from those age groups.

DISCUSSION

Because of the various difficulties previously discussed, the specific prevalence rates which we found in the present study cannot be easily compared with those published by most other investigators. Nevertheless, we can compare certain trends in our sample with those reported elsewhere.

Published studies disagree as to whether there is a difference in prevalence of antibodies between males and females. Beverley *et al.* (1954) have found, as we did in the present series, that there is little difference between the sexes with regard to prevalence of positive titers. Chi-square tests on their data, derived from 581 "normal" persons of all ages in Sheffield, England, showed no significant difference between males and females. Vivell and Buhn (1953) tested 54 healthy, pregnant women, 132 healthy, non-pregnant women, and 70 healthy men in Germany, and likewise found no significantly higher prevalence of positive titers in either sex. On the other hand Harboe (1952) studied 1,599 blood donors

in Oslo, Norway and found a higher percentage of positive women than men in all age groups. (Titers below 1:40 were considered negative.) In a later reference to this work, Harboe (1953) stated that "the women showed significantly higher titers than the men." Iversen (quoted by Hein, 1952) tested 180 persons (presumably in Hamburg, Germany) and found no difference between the sexes at a titer of 1:25, whereas twice as many females as males had titers of 1:50 or higher. It is not clear whether this was a random, unselected population. Kudicke and Pöhlig (1954) reported tests on 3,428 sera from Lower Saxony, which showed a strong prevalence of positive tests in the female sex beginning with the second decade of life. As these authors point out, however, their material was sent in by physicians for diagnostic testing and included both healthy and sick persons; many were cases of suspected toxoplasmosis. With this in mind, it would be most hazardous to consider this series as indicating a higher prevalence of positive reactions in females of the general population.

Nearly all investigators have found, as we did in the present study, that the prevalence of positive reactors increases in successive age groups of the population. Feldman (quoted by Weinman, 1952) found a steady increase in positive sera (titer 1:16 or higher) from 5 per cent positive at ages 0–4 years to 65–70 per cent positive at ages 40–60 and above. Comparable findings have been reported by Keller and Vivell (1952) in 1,024 persons tested in Freiburg; by Kudicke and Pöhlig (1954) in 3,428 sera from Lower Saxony; and by Thalhammer (1953, 1955) in 1647 sera from Vienna. In contrast to these findings, the report of Beverley *et al.* (1954) states that they could find "no significant difference at a titer of ¼ or more between the age groups from 20 onwards" in 581 normal persons in Sheffield, England. Harboe (1952) likewise found no increase in the prevalence of positive titers with increasing age among 1,599 blood donors in Oslo. Unfortunately, these sera were tested only at titers of 1:40 or higher, whereas we have shown that there is a shift in modal titer with increasing age and that adult sera tend to be positive at titer levels below that chosen by Harboe (see Fig. 2). Harboe has thus excluded the substantial number of sera which give positive reactions at titers of 1:16 or lower, and the prevalence rates which he derives from his series therefore are not comparable to ours or to those reported in the other studies mentioned above.

The shift in modal titer previously described and again mentioned with reference to Harboe's data, has also been strongly emphasized by Beverley *et al.* (1954). The chi-square test on their data from 581 sera in Sheffield, England, showed significant difference between the age groups ≥ 20 and the two lower groups at a titer of ¼ or more. The same tendency toward higher titers in the younger age groups is suggested in the findings reported by Feldman (1953) from a study of 270 sera from New Orleans and 293 from Portland, Oregon. Since the data are presented as scatter graphs rather than numerically, it is difficult to make exact comparisons. Most other studies do not present sufficient data to permit analysis. However, Kudicke and Pöhlig (1954) present graphic analyses of their study in Lower Saxony which show no evidence of a shift in modal titer such as we have been discussing. Nevertheless, in discussing their results these

authors carefully point out that theirs was a mixed sample, containing sera of both healthy and sick persons. It is quite possible that inclusion of suspected cases of toxoplasmosis in their series has introduced an abnormally large number of sera with high titers, thus masking the titer distribution seen in a normal, unselected population.

The bimodal distribution of titers which we found in the present series (Fig. 3) has not been described by other authors, probably because few have tested sera at the lower dilutions (such as 1:4) or undiluted. Feldman (1954) may have had this characteristic in mind when he stated that titers of 1:16 or above are considered significant, but the reasons for this statement are not given. We do not at present have any evidence regarding the possible significance of the unexpectedly large number of sera which we found positive at the undiluted level. Sera of several species of animals are known to contain a non-specific substance which interferes with the dye test at low dilutions (Sabin and Feldman, 1948), but this property is thermolabile and the inactivation at 56° C. for 30 minutes which all sera routinely receive in our tests would have destroyed any similar substance in our specimens. Addition of merthiolate as a preservative may occasionally produce false-positive reactions in undiluted sera (unpublished data from our laboratory) but this possibility can be excluded in the present case since no preservatives were used.

Although there is no satisfactory evidence that any other infectious agent has a common antigen with *Toxoplasma gondii*, or that the antibody measured by the dye test can result from anything but toxoplasmic infection, the possibility of non-specific cross reactions cannot be entirely ignored at the present time. Such a possibility was suggested by Mülhpfordt (1951), who reported that dye test titers rose from negative to positive at dilutions as high as 1:400 in guinea pigs, rats, and hamsters after only one feeding with sarcosporidia (*Sarcocystis tenella* and *S. miescheriana*). Mühlpfordt also reported positive dye tests in sheep naturally infected with *Sarcocystis*. Awad and Lainson (1954) found the dye test irregularly positive in sheep harboring *Sarcocystis*, but serum from a histologically-proven human case of sarcosporidiosis was negative, as was that from a guinea pig artificially immunized to *Sarcocystis*. Varela *et al.* (1955) have recently reported a considerably higher proportion of positive dye tests in *Sarcocystis*-infected rats (*Rattus norvegicus*) than in the general rat population of Mexico City. These apparently divergent findings indicate that the relation of *Sarcocystis* infection to the Sabin-Feldman dye test needs additional study and critical evaluation.

Michalzik (1953) suggested that women infected with *Trichomonas vaginalis* have positive dye tests more frequently than the general population, but his comparison is based on a general population derived from several different areas of Germany, tested in various laboratories using somewhat dissimilar test procedures, and reported without regard to age. His conclusions are therefore open to doubt. Awad (1954) states that mice and rabbits inoculated with bacteria-free cultures of *Trichomonas vaginalis* develop a cytoplasm-modifying antibody which, 3–5 weeks after inoculation, gives positive dye test reactions in

dilutions as high as 1:512. If human infection with this parasite leads to false positive reactions in the dye test, it is difficult to understand why we, as well as a number of other investigators, have failed to find a significantly higher proportion of positive tests in the female sex. Awad (1954) also reports an experiment in which four mice recovering from infection with *Trypanosoma cruzi* had positive dye tests at titers of 1:256 or higher when tested 68 days after infection. Varela *et al.* (1955) recently substantiated this finding with a report that positive dye tests (titers 1:32 or higher) were found in 13 out of 16 white laboratory rats previously inoculated with *T. cruzi*. There clearly is need for additional critical investigation of the relationship of human flagellate infections to the Sabin-Feldman dye test.

Only four other investigations of presumably "normal" populations (Verlinde and Makstenieks, 1951; Keller and Vivell, 1952; Beverley *et al.*, 1954; Thalhammer, 1955) have been reported in sufficient detail to justify the comparison of prevalence rates with those which we found and which are given in Table 5. In Figure 4 we have compared our results with the four studies just mentioned. In general, the rates which we found are intermediate between the relatively low values found in England (Beverley *et al.*) and the higher values found in the Netherlands (Verlinde and Makstenieks); in Germany (Keller and Vivell); and in Austria (Thalhammer). We do not know whether these differences have an epidemiological significance. In fact, it would be unwise to compare even these five studies too critically, inasmuch as identical dilution series were not used in all five studies, undiluted sera were not tested in all of them, and there may be other differences in technique or in the selection of sera. As a further point of difference it should be pointed out that all of our sera came from Negroes, whereas the sera in the other studies presumably were from Caucasians.

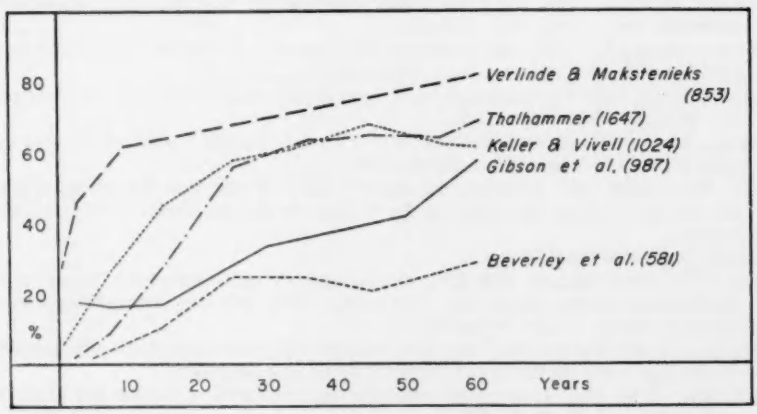


FIG. 4. Graphic comparison of the prevalence of positive titers from our series with four other published reports. The number of sera tested in each study is given in parentheses. (Adapted from Thalhammer, 1955).

SUMMARY

Using the Sabin-Feldman "dye test", 987 sera from the rural, Negro population of Fayette County, Tennessee, were tested for antitoxoplasmic antibodies. The following conclusions were established:

1. There is no significant difference between the sexes at any age with regard to anti-toxoplasmic antibodies.

2. With the exception of the youngest age group, the percentage of positive titers in the population increases with age.

3. There is a highly significant shift of modal titer, from positive at a dilution of 1:64 or higher in persons under 20 years of age to 1:16 or less in persons 20 years of age or more.

4. Considerable evidence can be seen of a bimodal distribution of titers, with an abnormally large number of undiluted positive sera.

5. When the data for each age group are standardized to the actual census population, the overall prevalence of positive reactors is 29.9 per cent for all positive titers, and 21.8 per cent for titers of 1:16 or higher.

REFERENCES

AWAD, F. I., 1954. The diagnosis of toxoplasmosis. Lack of specificity of Sabin-Feldman dye test, *Lancet* **267**(6847): 1055–1056.

AWAD, F. I., AND LAINSON, R., 1954. A note on the serology of sarcosporidiosis and toxoplasmosis, *J. clin. Path.* **7**: 152–156.

BEVERLEY, J. K. A., BEATTIE, C. P., AND ROSEMAN, CISSIE, 1954. Human Toxoplasma infection, *J. Hyg., Lond.* **52**: 37–46.

EYLES, DON E., JONES, FRANCES J., AND SMITH, CLINTON S., 1953. A study of *Endamoeba histolytica* and other intestinal parasites in a rural West Tennessee community, *Am. J. Trop. M. Hyg.* **2**: 173–190.

FELDMAN, HARRY A., quoted by Weinman, 1952, *q.v.*

FELDMAN, HARRY A., 1953. The clinical manifestations and laboratory diagnosis of toxoplasmosis, *Am. J. Trop. M. Hyg.* **2**: 420–428.

FELDMAN, HARRY A., 1954. Laboratory methods in current use for the study of toxoplasmosis, *Adv. Ophthal.* **3**: 1–11. Karger, Basel and New York.

HARBOE, ARILD, 1952. Toxoplasma dye test titers of 1600 blood donors in Oslo, *Acta path. microb. scand., Suppl.* **93**: 325–331.

HARBOE, ARILD, 1953. Investigations in Norway on toxoplasmosis. *Atti del VI Congresso Internazionale di Microbiologia, Roma,* **5** (Sec. 15): 467–469.

HEIN, WULF, 1952. Das Verhalten des Sabin-Feldman-Testes und der Westphal'schen Komplementbindungs-Reaktion bei der Lungentuberkulose, *Zschr. Tropenmed.* **3**(3): 339–357.

IVERSEN, quoted by Hein, 1952, *q.v.*

KELLER, W., AND VIVELL, O., 1952. Über die klinische und epidemiologische Bedeutung des Antikörpernachweises gegen das *Toxoplasma gondii* mit dem Sabin-Feldmanschen Farbtest, *Ztschr. Kinderh.* **71**: 42–60.

KUDICKE, H., AND PÖHLIG, W., 1954. Erfahrungen und statistische Betrachtungen über den Serofarbtest nach Sabin und Feldman, *Zschr. Hyg.* **140**: 350–371.

MICHALZIK, KURT, 1953. Trichomonas vaginalis und positive Seroreaktion auf Toxoplasmose, *Dtsch. med. Wschr.* **78**: 307.

MÜHLPFORDT, HEINZ, 1951. Das Verhalten Sarcosporidien-infizierter Tiere im Sero-Farbtest auf Toxoplasmose nach Sabin-Feldman, *Zschr. Tropenmed.* **3**: 205–215.

SABIN, ALBERT B., AND FELDMAN, HARRY A., 1948. Dyes as microchemical indicators of a

new immunity phenomenon affecting a protozoon parasite (*Toxoplasma*), *Science* **108**(2815): 660–663.

THALHAMMER, O., 1953. Wert und Grenzen der Toxoplasmoseteste, *Zschr. Immunforsch.* **110:** 361–374.

THALHAMMER, O., 1955. Die erworbene Toxoplasmose. Eine kritische Übersicht, *Wien. Zschr. inn. Med.* **36:** 1–29.

VARELA, GERARDO, ROCH, EUSTAQUIO, AND VÁZQUEZ, ARMANDO, 1955. Virulencia, cultivo, polisacáridos, toxinas y la prueba colorante, estudiados con una cepa de *Toxoplasma gondii*, *Rev. Inst. salubr. enferm. trop., Méx.* **15:** 73–80.

VERLINDE, J. D., AND MAKSTENIEKS, O., 1951. Toxoplasmosis in Nederland, *Ned. tschr. geneesk.* **95:** 2050–2059.

VIVELL, O., AND BUHN, WERNER H., 1953. Zur Problem der Toxoplasmose in der Gravidität. Ergebnisse serologischer Untersuchungen bei fertilen Frauen sowie Stellungnahme zur Aureomycin-prophylaxe der latenten Toxoplasmose, *Aerztl. Forsch.* **7:** 326–335.

WEINMAN, DAVID, 1952. Toxoplasma and toxoplasmosis, *Annual Rev. Microb.* **6:** 281–298.

FINAL REPORT ON THE FIRST USE OF PUROMYCIN FOR THE TREATMENT OF SLEEPING SICKNESS

CARLOS TRINCÃO, A. R. PINTO, ALMEIDA FRANCO AND ALFREDO NOGUEIRA

Department of Hematology and Protozoology of the Institute of Tropical Medicine, and the Missão de Estudo e Combate à Doença do Sono na Guiné Portuguesa

In a previous paper (Trincão *et al.*, 1955), the results of the treatment of 15 patients with the antibiotic Puromycin[1], were reported, as observed 6 months after the end of treatment. In every case the trypanosomes disappeared from the blood stream and from the lymph gland juice within 48 hours, and blood cultures became negative for trypanosomes 72 hours after the beginning of the treatment. Nevertheless, 4 of the 15 patients had relapsed when examined after 6 months (Cases 4, 9[2], 11 and 12). The others were in good clinical condition, and no trypanoeomes were found in their blood or lymph gland juice.

The 11 clinically cured patients continued under observation without any other specific therapy, and further examinations were made 12, 17 and 25 months after the end of treatment. These consisted of routine clinical observations, determination of the cell and protein content of the cerebrospinal fluid, and search for trypanosomes, but after 12 months only Case 2 remained suspicious. The search for trypanosomes was consistently negative as in the other cases following the 48-hour examination.

The results of the cerebrospinal fluid examination are given in Table 1. The improvement is extremely significant since, if the drug had not been effective, all the cases, in the absence of any other specific therapy, would have progressed to the nervous stage of the disease within 25 months, with highly abnormal cerebrospinal fluids. Moreover, in the patients whose spinal fluid was abnormal before treatment (Cases 3, 10, 14 and 15), it became normal afterwards, the only exception being Case 2. This patient, when examined 17 months after treatment, had an abnormal cerebrospinal fluid. Serology for syphilis being negative, this was considered a relapse despite the fact that no trypanosomes could be found in repeated searches of the blood and lymph gland juice. The patient, who was then 8 months pregnant, was therefore treated with tryparsamide.

The advantages of an antibiotic which is effective against sleeping sickness when administered by mouth in a short course of treatment, are obvious. Puromycin certainly deserves further trials.

Regarding the relapses, it may be pointed out that, except for Case 2, all occurred in patients who had reached the nervous stage of the disease. A series of cases in this stage is now being treated with Puromycin in order to ascertain its effectiveness in such advanced cases of sleeping sickness.

[1] Puromycin, now called Stylomycin, was very kindly supplied by the Lederle Laboratories.

[2] Not case 10 as was stated by mistake in the previous paper.

TABLE 1

Influence of Puromycin on parasites and cerebrospinal fluid in cases of sleeping sickness which did not relapse within six months of treatment

Cases	Total Doses gr.	Duration of Treatment in Days	Parasites in Blood or Lymph		Cerebrospinal Fluid									
			Before treatment	After treatment	Before treatment		At 6 months		At 12 months		At 17 months		At 25 months	
					Cells	Prot.	Cells	Prot.	Cells	Prot.	Cells	Prot.	Cells	Prot.
1	21	10	Pos.	Neg.	3	0,24	4	0,28	3	0,25	4	0,25	1	0,25
2	13,5	7	Pos.	Neg.	4	0,20	49	0,22	7	0,22	38	0,40	—	—
3	13,5	7	Pos.	Neg.	25	0,25	7	0,25	4	0,22	4	0,22	1	0,22
5	13,5	7	Pos.	Neg.	4	0,20	4	0,20	4	0,20	8	0,20	1	0,15
6	13,5	7	Pos.	Neg.	6	0,22	25	0,25	4	0,22	3	0,22	1	0,15
7	13,5	7	Pos.	Neg.	7	0,24	15	0,25	4	0,22	2	0,22	1	0,22
8	13,5	7	Pos.	Neg.	2	0,20	5	0,24	4	0,22	2	0,22	3	0,22
10	11,5	7	Pos.	Neg.	10	0,22	11	0,22	3	0,20	1	0,16	2	0,20
13	9,5	10	Pos.	Neg.	2	0,22	8	0,25	4	0,22			2	0,20
14	9,5	10	Pos.	Neg.	15	0,24	7	0,30	7	0,25	2	0,22	3	0,25
15	9,5	10	Pos.	Neg.	10	0,20	6	0,22	6	0,22	2	0,22	3	0,20

SUMMARY

Of 15 cases of sleeping sickness treated with Puromycin, 5 relapsed and 10 were free of infection, clinically and parasitologically, 25 months after treatment. No other specific drug was administered at any time to any of the 10 patients who did not relapse.

REFERENCE

TRINCÃO, C., ALMEIDA FRANCO, NOGUEIRA, A., PINTO, A. R., AND MUHLPFORDT, H., 1955. First report on the treatment of sleeping sickness with Puromycin, *Am. J. Trop. Med. & Hyg.* **4**: 13–17.

THE INCIDENCE OF *TRICHOMONAS VAGINALIS* IN THE VARIOUS AGE GROUPS

LOUIS G. FEO

Department of Obstetrics and Gynecology, Jefferson Medical College Hospital, Philadelphia, Penna.

Numerous investigators have made surveys of the prevalence of *Trichomonas vaginalis* in women attending antenatal or gynecologic clinics (Karnaky, 1946; Reich *et al.*, 1947; Johnson *et al.*, 1948; McKim and McCullagh, 1948; Morton, 1948; Jírovec and Peter, 1950; Perin *et al.*, 1951; Zaidman, 1951; Donald, 1952; Feo, 1953). Reports of the incidence of this flagellate in the different races have been published (Chippaux and Chippaux-Mathis, 1944; Mandoul and Fleurette, 1950). But the data refer almost exclusively to vaginal infections in women in the child-bearing age. Very little can be found in the literature about its occurrence in other age groups and in the general population (Jírovec *et al.*, 1942; Trussell, 1947; Whittington, 1951; Shaw *et al.*, 1952). The incidence of this parasite in infants, children and postmenopausal women is unknown. The present survey attempts to determine the frequency of occurrence of *Trichomonas vaginalis* in asymptomatic and normal infants, children, and non-pregnant women of various ages.

MATERIAL AND METHODS

Women attending the Medical Clinic of the Jefferson Medical College Hospital, newborns, and children attending the Pediatric Clinic supplied material for this study. A total of 1,119 patients were examined, of which 466 were white adults and 653 were Negroes, including 393 adults, 158 children and 102 infants.

The race, age, presence or absence of menses were recorded for each patient and all were closely questioned as to the presence of a vaginal discharge. In some women there seemed to be little correlation between the subjective interpretation of the presence or absence of a discharge and the objective findings. This was true especially of the Negro group. Two factors appeared responsible for this: the hygienic training of the patient and the employment of douches. Lavage is a common practice and many women suffering from a slight or moderate vaginal discharge are rendered symptom-free by its use. Therefore all patients with genital symptoms or employing frequent douches, and others revealing obvious gynecologic disorders upon examination, were excluded from the final analyses. No patient of menopausal age, in either race group, was under estrogen therapy.

Samples of vaginal exudates collected on cotton swabs were placed immediately in test tubes containing physiologic saline solution. A small drop of this mixture was then examined microscopically for trichomonads. Both the low and the high dry objectives were used. One wet smear examination was made of each patient but cultures were not done. Previous experience has shown that

no greater accuracy was obtained by the latter method provided a wisp of cotton was used for the swab and no more than 0.2 ml. of physiologic saline solution employed.

RESULTS

T. vaginalis was present in 37 (12.3 per cent) of 301 normal Negro women aged 20 to 81 years. The frequency of infection in the various age groups is shown in Table 1. The incidence of parasitism among the sexually active and child-bearing group of women (20–49 years) was 14.8 per cent with an incidence of 19.2 per cent among the 40 to 49 year old women. It is to be noted that the percentage of positive cases in women who have passed the menopause is unexpectedly high.

The data obtained in the group of normal white patients is shown in Table 2. In contrast to the adult Negro series the infection was more uniformly distributed among the various age groups of white women. Between the ages of 20 and 92 years 13 (2.9 per cent) of 439 individuals examined were positive for *T. vaginalis*. The incidence of infection was 3.5 per cent among the women aged 20 to 49 years. The absence of the flagellate in 139 patients aged 60 to 69 years is unexplained. However in the 70 to 79 year old group 2 (2.9 per cent) of 68 women harbored the parasite.

A series of 158 Negro children and young adults was studied and of these 24 were found positive for *T. vaginalis*. On further subdivision into age groups it was found that of the 84 children aged 1 to 9 years, 3 (aged 5, 6, and 8 years) carried the flagellate, an incidence of 3.6 per cent. Of the 74 Negro girls aged 10 through 19 years 21 showed the organism. Seven of these patients, however, aged 11, 13, 14, 15 and 19 (3 cases) years, presented a moderate to profuse vaginal discharge on examination and were excluded from further study. Therefore an incidence of 18.9 per cent was recorded. One of the girls remaining in the series was 12 years old without menses. The others had all menstruated and were aged 12 years (3), 13 years (2), 14 years (2), 15 years (3), 16 years (2) and 17 years (1). No trichomonads could be demonstrated in a group of 102 Negro newborns aged 1 to 5 days.

TABLE 1

Incidence of Trichomonas vaginalis *in normal Negro women*

Age	Total No. examined	Cases Excluded	No. Normal Women	No. with *T. vaginalis*	Absolute Incidence %
20–29	43	5	38	4	10.5
30–39	52	19	33	3	9.1
40–49	106	28	78	15	19.2
50–59	86	27	59	6	10.2
60–69	82	11	71	6	8.5
70–79	23	2	21	3	14.3
80–89	1	0	1	0	0
Total.....	393	92	301	37	12.3

TABLE 2

Incidence of Trichomonas vaginalis *in normal white women*

Age	Total No. Examined	Cases Excluded	No. Normal Women	No. with *T. vaginalis*	Absolute Incidence %
20–29	42	4	38	1	2.6
30–39	57	3	54	2	3.7
40–49	55	3	52	2	3.8
50–59	85	4	81	6	7.2
60–69	152	13	139	0	0
70–79	68	0	68	2	2.9
80–89	6	0	6	0	0
90–99	1	0	1	0	0
Total.....	466	27	439	13	2.9

It is unfortunate that parental interference and in many instances lack of co-operation from the patients rendered impossible a study of a comparable series of white children.

DISCUSSION

It is a clinical observation that many women with a slight to moderate vaginal discharge are rendered asymptomatic by the simple expedient of douching. This is true also of many women who harbor a *T. vaginalis* infection and suffer no discomfort. As a result it is difficult to arrive at an incidence of parasitism in the so-called normal clinic patient. If lavage is considered an obligatory practice among the women employing the douche and all such are eliminated, then the present data will give an absolute incidence of *T. vaginalis* infection of 2.9 per cent and 12.3 per cent in the white and Negro groups of normal adult women, respectively. The frequency of infection during the sexually active and child-bearing ages (20–49 years) was 3.5 per cent and 14.8 per cent respectively. These figures for the white women are considerably less than those reported by Jírovec *et al.* (1942) for healthy women (14.3 per cent), with a high incidence of 23.7 per cent among women aged 20–49 years. However in a very carefully checked sample of the general population, Whittington (1951) found that the infection was present in 3.4 per cent of 507 normal white women. The similarity of results in the latter survey with the present study among white women is striking.

The vaginal infection in younger individuals and infants has received but scant mention in the literature. The actual incidence of parasitism is unknown. The literature reports totals of four cases in the 1 to 9 year group, and 53 cases in children 10 to 16 years of age have been reported (Frankenthal and Kobak, 1933; Ayer and Neil, 1934; Karnaky, 1936; Brady and Reid, 1942; Drabløs, 1952; Schauffler, 1953). Many of these patients however were symptomatic. In the present survey only 3 of 84 Negro girls examined between the ages of 1 and

9 years were positive but there were 14 among 74 Negro girls aged 12 to 17 years.

Involvement in early life has been noted in 5 cases, ranging in age from 8 days to 6 weeks (Brady and Reid, 1942; Hersh and Hamilton, 1942; Trussell and Wilson, 1942; Drabløs, 1952). This infrequent occurrence of *T. vaginalis* in infants and young patients is emphasized by the observations in this study. The organisms could not be demonstrated in 102 Negro newborns. No doubt this is due to the meticulous partum and postpartum care patients receive from present day obstetric services.

A few scattered reports indicate that *T. vaginalis* has been seen in women who have passed the menopause (Geiger, 1935; Mohr, 1937; Jírovec *et al.*, 1942; Donald, 1952; Shaw *et al.*, 1952), although Lang and Rakoff (1953) did not note any cases beyond the age of 60. This supposedly rare infection in the menopausal woman is attributed to the waning function of the estrogens. However this is contrary to recent observations by the author. In the latter study, of the women examined aged 60 years and over, 24 were positive, and yet all of these patients showed marked deficiency in estrogen effect. In this same age group 2 of 214 normal white women and 9 of 93 normal Negro women were positive. Rather it is the considered opinion of the author that the important factor, for the implantation and survival of *T. vaginalis* in the vagina, is the presence of serum, whether derived from the uterus, cervix or the vagina.

SUMMARY

The incidence of *Trichomonas vaginalis* in a group of 301 normal Negro women aged 20 to 81 years was 12.3 per cent and 14.8 per cent for those between the ages of 20 and 49 years. For the respective groups of normal white women the percentage incidence was 2.9 and 3.5.

The frequency of infection in women of the Negro group who have passed the menopause (60 years and over) was relatively high in the Negro group though lower than in the younger women. Of 93 normal Negro women examined 9 were positive, while 2 of 214 normal white patients harbored the flagellate. The incidence of infection was found to be higher among patients with symptoms.

No trichomonads could be demonstrated in a series of Negro newborns aged 1 to 5 days. The incidence of infection in normal Negro females 1 to 9 years old and between the ages of 10 and 19 years (actual age range 12–17 years) was 3.6 per cent and 18.9 per cent respectively.

REFERENCES

AYER, E. W., AND NEIL, J. M., 1934. Trichomonas vaginitis, *California and West. Med.* **40**: 117.

BRADY, L., AND REID, R. D., 1942. Treatment of *Trichomonas vaginalis* vaginitis with *Lactobacillus*, *Ann. Surg.* **115**: 840–848.

CHIPPAUX, C., AND CHIPPAUX-MATHIS, J., 1944. *Trichomonas vaginalis* in Togoland women, *Med. Trop. Marseilles* **4**: 269–300.

DONALD I., 1952. Etiology and investigation of vaginal discharge, *Brit. M. J.* **2:** 1223–1226.

DRABLØS, A., 1952. Infantile vulvovaginitis due to *Trichomonas vaginalis*, *Nord. Med.* **47:** 81–83.

FEO, L. G., 1953. Concurrent association of *Trichomonas vaginalis*, the filiform bacillus of Döderlein and *Syringospora* in pregnancy, *Am. J. Obst. & Gynec.* **65:** 1330–1333.

FRANKENTHAL, L. E., JR., AND KOBAK, A. J., 1933. Trichomonas vaginitis in children, *J.A.M.A.* **100:** 1746–1748.

GEIGER, W., 1935. Importance of *Trichomonas vaginalis* in biology of vagina, *Ztschr. f. Geburtsh. u. Gynäk.* **112:** 79–94.

HERSH, J. J., AND HAMILTON, R. C., 1942. *Trichomonas vaginalis* vaginitis in young infants, *Pennsylvania M. J.* **45:** 794.

JÍROVEC, O., BREINDL, V., KUČERA, K., AND ŠEBEK, V., 1942. Vaginal trichomoniasis, *Zentralbl. f. Bakt. (Abt. 1)* **148:** 338–358.

JÍROVEC, O., AND PETER, R., 1950. *Trichomonas vaginalis*, *Gynaecologia* **129:** 145–160.

JOHNSON, C. G., DOUGLAS, J. W., AND MAYNE, R. Y., 1948. Treatment of *Trichomonas vaginalis* with tyrothricin, *Am. J. Obst. & Gynec.* **56:** 184–186.

KARNAKY, K. J., 1936. A rare case of *Trichomonas intestinalis* in stool and *Trichomonas vaginalis* in the bladder and vagina of a 10-months-old child, *South. M. J.* **29:** 939–940.

KARNAKY, K. J., 1946. Trichomoniasis, a twelve year study, *West. J. Surg., Obst., and Gynec.* **54:** 61–64.

LANG, W. R., AND RAKOFF, A. E., 1953. Vaginitis in the menopausal and post-menopausal woman, *J. Am. Geriat. Soc.* **1:** 520–530.

MANDOUL, R., AND FLEURETTE, G., 1950. Vaginal trichomoniasis in Moslem women, *Bull. Soc. Path. Exot.* **43:** 607–615.

MCKIM, W., AND MCCULLAGH, H., 1948. Incidence, signs, symptomatology and special cases, *M. Press.* **219:** 469–471.

MOHR, H., 1937. Pathogenicity of *Trichomonas vaginalis*, *Ztschr. f. Geburtsh. u. Gynäk.* **115:** 115–129.

MORTON, J. H., 1948. *Trichomonas vaginalis* infestation in women, *M. Press.* **219:** 246–250.

PERIN, L., SISSMANN, R., AND SENGHOR, J., 1951. Occurrence of *Trichomonas vaginalis* in prostitutes, *Bull. Soc. Franç. Dermat. et Syph.* **58:** 364–367.

REICH, W. J., BUTTON, H. L., AND NECHTOW, M. J., 1947. Treatment of *Trichomonas vaginalis* vaginitis, *Surg., Gynec., and Obst.* **84:** 891–896.

SCHAUFFLER, G. C., 1953. *Pediatric Gynecology* 3rd ed., The Year Book Publishers, Inc., Chicago, Ill., p. 96.

SHAW, H. N., HENRIKSEN, E., KESSEL, J. F., AND THOMPSON, C. F., 1952. Clinical and laboratory evaluation of "Vagisol" in the treatment of *Trichomonas vaginalis* vaginitis, *West. J. Surg.* **60:** 563–570.

TRUSSELL, R. E., 1947. Trichomonas vaginalis *and Trichomoniasis*. Charles C Thomas, Springfield, Illinois, pp. 92–93; 124.

TRUSSELL, R. E., AND WILSON, M. E., 1942. Vaginal trichomoniasis, *Am. J. Obst. & Gynec.* **44:** 292–295.

WHITTINGTON, M. J., 1951. The incidence of *Trichomonas vaginalis* in a sample of the general population, *J. Obstet. Gynaec. Brit. Emp.* **58:** 398–405.

ZAIDMAN, H., 1951. Genital trichomoniasis in the female, *Presse Méd.* **59:** 1368.

POLIOMYELITIS IN LIBERIA

Prevalence of the Disease, Sero-Immunity Resulting from Subclinical Infection, and Indications for Prophylactic Vaccination[1]

HENRY M. GELFAND and MAX J. MILLER

Department of Tropical Medicine and Public Health, School of Medicine, Tulane University of Louisiana, New Orleans, La., and Liberian Institute of the American Foundation for Tropical Medicine, Harbel, Liberia

The recent emergence of poliomyelitis as an epidemic disease in tropical areas of the world (Gear, 1955) has led to increased interest in the patterns of immunity to polioviruses in these countries and in others which have not yet experienced epidemics. Relatively little has been published concerning sero-immunity to poliomyelitis in tropical Africa (south of the Sahara), although the disease appears to be endemic throughout the continent (Freyche *et al.*, 1955). Where virus isolations have been made and/or serum neutralizing antibody studies performed, polioviruses have been found (all three types for the continent as a whole), together with evidence of infection and the acquisition of active immunity early in life (Gear *et al.*, 1951; Pellissier, 1954; Placido De Sousa, 1954; Gear, 1955; Goffe, *et al.*, 1955; Freyche, *et al.*, 1955).

Very little is known about the incidence of poliomyelitis in Liberia. In the absence of morbidity statistics, the impressions of practicing physicians are the only basis for approximations. One of the authors (MJM) has been able to obtain impressions from two reliable sources. The Firestone Rubber Plantation, located in the forested region of the Central Province, provides medical care for approximately 50,000 employees and their dependents, with a ratio of foreign (white European and American) to Liberian staff in the order of 1:200. Both groups have consisted predominantly of young adult males without families, but there is a significant number of women and children also. Medical facilities for diagnosis and treatment are adequately available to both groups, and it is very unlikely that a significant case of *paralytic* poliomyelitis would go undetected. Nevertheless, during the past 10 years, there have been two such cases in the foreign adult population and not one in the African population. In Monrovia, the capital city of approximately 35,000 population, a European physician who has been a resident of Liberia for 26 years, and is well acquainted with the medical problems of the city, states that he has seen paralytic poliomyelitis in Liberians, but that it is very rare indeed. By contrast, in the relatively insignificant European population, he has encountered one paralytic case in a foreign missionary woman and a fatal case in a young German doctor.

Assuming the validity of these clinical impressions, it appears that poliomyelitis exists in Liberia, with a very low incidence of disease in the native African population, and a relatively high incidence in the white foreign population. This discrepancy in incidence rates is in agreement with observations made in other

[1] This study was aided in part by a grant from The National Foundation for Infantile Paralysis.

tropical areas (Sabin, 1949; Paul, 1949). It may be explained as the result of widespread dissemination of polioviruses in these areas, resulting in relatively benign infection and the acquisition of long-lasting immunity very early in life in the native population, in contrast with the relative deficiency in immunity in the adult European visitor (Paul, 1955; Melnick *et al.*, 1955). Sabin (1955) has offered a modification of this generally accepted theory by suggesting that the background of immunity possessed by populations in under-sanitated areas is produced by the more abundant strains of low virulence which are circulating freely, and which result in the protection of all but the very young (who have not yet been infected) from the occasional appearance of virulent strains. The foreign adult, not being so protected, is at hazard when the virulent strains do periodically appear.

It was to be expected, therefore, that sero-immunity to poliomyelitis would be very prevalent in the native Liberian population, and would be acquired at a very young age. This was already suggested by an early report of Hudson and Lennette (1933). Using the neutralization test in monkeys, these workers demonstrated antibodies against the virulent PMV strain (subsequently shown to be type II) in the blood of 18 of 19 adult Liberian males. To investigate the rate at which this sero-immunity is acquired, blood sera were obtained in August, 1955, from a group of 80 Liberians of various ages living in the vicinity of the Liberian Institute of the American Foundation for Tropical Medicine in Marshall Territory, Liberia. This is a rural area near the Atlantic coast about 30 air miles east of Monrovia, and is described in some detail elsewhere (Gelfand, 1955).

MATERIALS AND METHODS

Sera were obtained from a cubital vein of healthy donors with the use of vacuum bleeding tubes. These were stored unopened in the refrigerator for up to one week until sent by air express to the virus laboratory at the Tulane University School of Medicine. Up to two weeks elapsed between the bleeding in Liberia and the separation of serum from blood clot in New Orleans, but no serious hemolysis had occurred. The sera were inactivated at 56° C. for one-half hour, and then stored at −20° C. in screw-top vials until used.

The serum neutralization test was performed on all specimens at a final dilution of 1:4. Currently accepted tissue culture techniques using monolayer monkey kidney cell films (Melnick, 1955) were employed. Approximately 100 tissue culture doses were used of each of three prototype poliovirus strains as described elsewhere (Bhatt *et al.*, 1955).

RESULTS

Table 1 shows the results of screening neutralization tests against the three poliovirus types according to the age of the donor. The column headed "All types" gives the average poliovirus infection experience by age on the assumption that infection with each virus type is an independent event (even if temporally simultaneous) Therefore each individual has three opportunities for infection, and the percentage is calculated by adding together all positive results as the

TABLE 1

Sero-immunity to polioviruses in healthy Liberians in Marshall Territory, Liberia, as indicated by neutralization test

Age (yrs.)	Number of Sera	Sera with Neutralizing Antibody to Indicated Virus Type						Mean per cent for all 3 types*
		I		II		III		
		Number	Per cent	Number	Per cent	Number	Per cent	
1–2	7	0	0	2	29	5	72	34
3–4	7	1	14	5	71	5	72	52
5–6	9	7	78	9	100	8	89	89
7–9	14	13	93	13	93	12	86	91
10–19	28	27	96	28	100	24	86	94
20–29	6	4	67	5	83	6	100	83
30+	9	8	89	9	100	8	89	93

* See text for method of computing.

numerator, and multiplying the number of persons by three for the denominator. (The same result is obtained more simply by averaging the percentages for each of the three types).

DISCUSSION

It is evident that poliovirus is present in Liberia, and is represented by strains of types I, II and III. Infection occurs at a very early age and nine-tenths of the infections that can occur have already taken place by the age of 5–6 years. Even though the number of specimens is small, the more rapid accumulation of immunity to type III as contrasted with type I is marked. Since the relative deficiency in type I is limited to children up to the age of 4 years, it suggests that type I strains have not been active in this community for about that period of time, whereas type III has been present and actively disseminating during the past year. This is very similar to the situation observed by us (Fox *et al.*, 1956) in southern Louisiana in 1953–4, when type III was relatively deficient. It was followed by a preponderance of subclinical type III infections in the 1954 "polio season", resulting in a slight relative deficiency in type I by the end of the year. This in turn was followed by a preponderance of type I infections in the 1955 polio season. It might be anticipated, therefore, that a wave of type I infection will occur in this Liberian community in the near future, when it is introduced into the non-immune, young population.

In Table 2 and Fig. 1 the average poliovirus experience, as indicated by serum neutralization test, of this Liberian community is compared with that in a very under-sanitated, primarily urban population in and near Casablanca, Morocco (Paul and Horstman, 1953), a poorly sanitated Negro population in urban and rural southern Louisiana (Fox *et al.*, 1956) and a mixed population including both upper and lower socio-economic levels, and both Negro and white groups in Winston-Salem, North Carolina (Melnick and Ledinko, 1953). The marked similarity of the Liberian and Louisiana curves is striking. The extremely poor

TABLE 2

Comparative sero-immunity to polioviruses (all types) in Liberia, French Morocco, Southern Louisiana (Negroes), and Winston-Salem, North Carolina

Ages (years)	Per Cent Positive			
	Liberia	French Morocco*	Southern La. Negroes†	Winston-Salem, N. C.‡
1	—	43	—	—
1–2	34	—	32	9
2–4	—	75	—	—
3–4	52	—	47	14
5–9	—	91	—	—
5–6	89	—	78	40
7–9	91	—	86	42
10–14	—	—	—	46
10–19	94	95	93	—
20+	89	91	95	—

* Based on data presented by Paul & Horstman (1955).
† Based on data presented by Fox, et al. (1956).
‡ Based on data presented by Melnick & Ledinko (1953).

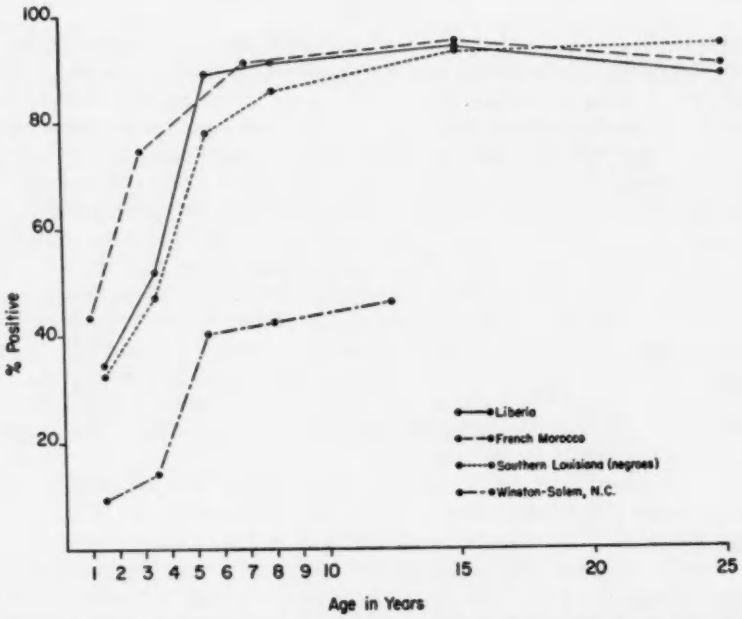


FIG. 1. Comparative sero-immunity to polioviruses (all types) in Liberia, French Morocco, Southern Louisiana (negroes), and Winston-Salem, N. C. Based on data adapted from Paul and Horstmann (1955), Fox et al (1956), and Melnick and Ledinko (1953).

sanitary status and more crowded urban conditions in Casablanca have resulted in an even more rapid accumulation of sero-immunes. In striking contrast is the situation in Winston-Salem where sero-immunity is acquired at a much slower rate. If this group is at all representative of the population of Americans from whom individuals are drawn to go to Liberia and other tropical areas, the increased risk of poliomyelitis experienced by these individuals is not difficult to understand.

The data and analysis presented above suggest that the mass inoculation of the indigenous Liberian population with poliomyelitis vaccine, as has been considered (personal communication), is not justified at the present time. However, the use of this vaccine in foreign visitors to Liberia might be considered.

SUMMARY

Clinical poliomyelitis is uncommon in the native population of Liberia, but is relatively much more frequent in foreign visitors. The serum neutralization test against poliomyelitis viruses was performed, using the serum of 80 Liberians of various ages, and indicated a rapid accumulation of sero-immunity, up to an average level of about 90 per cent by 5–6 years of age. This pattern of sero-immunity is very comparable to the patterns found in other studies of under-sanitated areas, and indicates an abundance of poliovirus and infection at a very early age.

The pattern of sero-immunity in Liberia is markedly different from that in an American city studied by Melnick and Ledinko and perhaps typical of the population of foreigners in Liberia. This suggests that the foreign adult visitors may include a significant number of non-immunes who are at hazard in an under-sanitated area. The use of prophylactic vaccination against poliomyelitis is not now justified in the native Liberian population, but may be in the foreign visitor to Liberia.

REFERENCES

Bhatt, P. N., Brooks, M., and Fox, J. P., 1955. Extent of infection with poliomyelitis virus in household associates of clinical cases as determined serologically and by virus isolation using tissue culture methods, *Am. J. Hyg.* **61:** 287–301.

Fox, J. P., Gelfand, H. M., LeBlanc, D. R., and Conwell, D. P., 1956. A continuing study of the acquisition of natural immunity to poliomyelitis in representative Louisiana households, *Am. J. Pub. Health* **46:** 283–294.

Freyche, M. J., Payne, A. M. M., and Ledessey, C., 1955. Poliomyelitis in 1953, *Bull. W.H.O.* **12:** 595–649.

Gear, J. H. S., 1955. Poliomyelitis in the under-developed areas of the world, *W.H.O. Monograph Ser.* No. 26: 31–58.

Gear, J. H. S., Measrock, V., Bradley, J., and Faerber, G. I., 1951. Poliomyelitis in South Africa. Studies in an urban native township during a non-epidemic year, *South African Med. J.* **25:** 297–301.

Gelfand, H. M., 1955. *Anopheles gambiae* Giles and *Anopheles melas* Theobald in a coastal area of Liberia, West Africa, *Trans. Roy. Soc. Trop. Med. & Hyg.* **49:** 508–527.

Goffe, A., Dick, G. W., and Pellissier, A., 1955. The similarity of Brazzaville encephalomyelitis virus and type 1 poliomyelitis virus, *Trans. Roy. Soc. Trop. Med. & Hyg.* **49:** 95–96.

HUDSON, N. P., AND LENNETTE, E. H., 1933. Neutralization of poliomyelitis virus by the serum of Liberian negroes, *Am. J. Hyg.* **17:** 581–586.

MELNICK, J. L., 1955. Tissue culture techniques and their application to original isolation, growth, and assay of poliomyelitis and orphan viruses, *Ann. N. Y. Acad. Sci.* **61:** 754–773.

——— AND LEDINKO, N., 1953. Development of neutralizing antibodies against the three types of poliomyelitis virus during an epidemic period, *Am. J. Hyg.* **58:** 207–222.

———, PAUL, J. R., AND WALTON, M., 1955. Serologic epidemiology of poliomyelitis, *Am. J. Pub. Hlth.* **45:** 429–437.

PAUL, J. R., 1949. Poliomyelitis attack rates in American troops, 1940–1948, *Am. J. Hyg.* **50:** 57–62.

——— 1955. Epidemiology of poliomyelitis, *W.H.O. Monograph Ser.* No. 26: 9–27.

——— AND HORSTMAN, D. M., 1955. A survey of poliomyelitis virus antibodies in French Morocco, *Am. J. Trop. Med. & Hyg.* **4:** 512–524.

PELLISSIER, A., 1954. Enquête serologique sur l'incidence des virus neurotropes en Afrique Équitoriale Française, *Bull. Soc. Path. Exot.* **47:** 223–227.

PLACIDO DE SOUSA, C., 1954. Identifição, em culturas de tecidos, de vírus poliomyelíticos de Angola. (Identification in tissue cultures of the poliomyelitis virus found in Angola.), *J. Soc. Cienc. méd. Lisboa* **118:** 469–476.

SABIN, A. B., 1949. Epidemiologic patterns of poliomyelitis in different parts of the world. In: *Poliomyelitis.* Papers and Discussions presented at the First International Poliomyelitis Conference. Philadelphia, Lippincott.

——— 1955. Immunity in poliomyelitis with special reference to vaccination, *W.H.O. Monograph Ser.* No. 26: 297–334.

ISOLATION OF THE VIRUS OF ILHÉUS ENCEPHALITIS FROM MOSQUITOES OF THE GENUS *PSOROPHORA* CAPTURED IN HONDURAS

ENID DE RODANICHE

Gorgas Memorial Laboratory, Panama, R.P.

During the course of investigation to determine the vector of sylvan yellow fever in Honduras, an unidentified neurotropic virus was isolated from mosquitoes of the genus *Psorophora* (Rodaniche, 1956). On the basis of cross-immunity and neutralization tests it was determined that this virus was not that of yellow fever. Unfortunately, other appropriate virus strains or immune serums were not available here for making a final classification. However, Dr. Jordi Casals, of the Rockefeller Institute, generously offered to carry out systematic immunological studies with a pooled mouse hyper-immune serum forwarded to his laboratory, and found the virus to be "identical with or closely related to Ilhéus." Animal pathogenicity and other properties of the Honduran strain as observed in this laboratory also conform to those of Ilhéus.

The virus of Ilhéus encephalitis was isolated for the first time in 1944 by Laemmert and Hughes (1947) from a pool of mosquitoes of the genera *Aedes* and *Psorophora* captured in the vicinity of Ilhéus in the State of Baia, Brazil. *Aedes serratus* and *Psorophora ferox* were the predominant species in the pool. No further isolations of this virus were recorded until 1954 when it was encountered in Honduras and Trinidad, B.W.I. Anderson, Aitken and Downs (1956) reported two isolations from mosquitoes in Trinidad, one from a pool of *Psorophora* including 3 species. *P. ferox*, *P. cingulata* and *P. albipes*, and the other from a pool of 8 different genera but also including *P. ferox* and *P. albipes*. In view of the limited data available at present concerning the Ilhéus virus it may be of interest to present further details concerning the isolation and characteristics of the Honduran strain.

ISOLATION

The virus was recovered from a suspension of 50 mosquitoes of the genus *Psorophora*, including principally *Psorophora ferox* but also *P. lutzii* and *P. varipes*. These mosquitoes were captured in the forested area near the village of La Masica on the north coast of Honduras on September 2, 1954, by Drs. H. Trapido and P. Galindo of the Gorgas Memorial Laboratory staff while carrying on field studies of the epidemiology of yellow fever in this area. Further details concerning the terrain and conditions of capture are given in the report by Trapido and Galindo (1955). The mosquitoes were received in the virus section of the laboratory in sealed tubes in dry ice and were stored in dry ice until such time as they could be inoculated into mice. A total of 5,657 *Psorophora* collected between August 7 and September 8, 1954, were received and tested in lots of approximately 50. Each lot was ground with 2 cc. of normal rhesus serum-saline

containing 600 Units of penicillin and 600 micrograms of streptomycin per cubic centimeter, centrifuged at 5000 r.p.m. during 15 minutes at a temperature of 5°C., and then the supernatant was injected intracerebrally into a group of 6 adult white Swiss mice, each mouse receiving 0.03 cc. Virus was recovered only once, from the batch of *Psorophora* previously mentioned.

PATHOGENICITY IN MICE

Laemmert and Hughes (1947) isolated the virus of Ilhéus encephalitis originally by the inoculation of mice with serum of a rhesus monkey receiving daily injections of suspensions of *Aedes* and *Psorophora*. The Honduran strain was readily recovered by direct intracerebral injection of adult Swiss mice, 6 of which were inoculated with the suspension of triturated *Psorophora* on July 19, 1955. Five became ill 8 to 13 days later. The sixth mouse remained asymptomatic but was subsequently found to be immune. Four mice were sacrificed for further passage and other studies, the fifth mouse recovered spontaneously. The virus has been readily maintained in mice for 55 consecutive passages to date. After the second passage the intracerebral injection of 10 per cent brain suspensions proved uniformly fatal. As the passages were continued, there was a progressive diminution in the length of the incubation period and increase in the virus titer in the brain and in the capacity of the virus to produce encephalitis when introduced by extraneural routes. From the 2nd to the 10th passage symptoms were first observed on the 5th to 6th day with death on the 6th to 8th. From the 11th passage to date symptoms have first been observed in the majority of adult mice on the 4th day with death on the 5th or 6th. After the 48th passage death occurred most commonly on the 5th day. Early symptoms are ruffling of the fur, arching of the back, and diminished activity. These are followed by tremors, ataxia, debility, paralysis, coma and death. Marked distention of the urinary bladder was noted at the time of death in most mice injected with early passage virus by the intracerebral route, but was not observed in mice receiving late passage brain suspensions. The LD_{50} of 10th passage virus was $10^{-4.2}$, of 38th passage virus $10^{-5.6}$ and of 44th passage virus $10^{-7.7}$. Both adult and infant mice developed uniformly fatal infections after intracerebral or intranasal inoculation with 10 per cent brain suspensions, the incubation period being about one day shorter in infant

TABLE 1

Susceptibility of mice to extraneural routes of inoculation in relation to number of passages of virus

Age of mice	Route of Inoculation	Number of Passages of Inoculum	Ratio between Mice Inoculated and Mice Dying
Adult (30–60 days)	Intraperitoneal	10	0/6
Adult (30–60 days)	Intraperitoneal	39	4/6
Adult (30–60 days)	Subcutaneous	10	0/6
Adult (30–60 days)	Subcutaneous	44	3/6
Infant (4 days)	Subcutaneous	10	0/10

than in adult mice and 2 days shorter after intracerebral than after intranasal inoculation. Adult mice failed to develop encephalitis or any other symptoms after subcutaneous or intraperitoneal injection with early passage virus as indicated in Table 1. However, encephalitis was readily produced by later passage virus, perhaps due to the significantly higher titers. Infant mice (4 days old) also failed to develop encephalitis after subcutaneous injection with early passage virus.

Virus was uniformly present in high titer in brain and cord tissue of animals showing nervous symptoms. It was recovered in low titer, less than 10^{-1}, in one experiment from 10 per cent suspensions by weight of the pooled spleens, lungs, kidneys, adrenal glands and heart muscle respectively of three adult mice sacrificed when semi-prostrate five days after intracerebral injection with 34th passage virus. This distribution is similar to that observed by Koprowski and Hughes (1946) after intraperitoneal inoculation although much higher titers were obtained by these authors. In one experiment virus was recovered from pooled blood of 4 adult mice on the 4th day after intracerebral injection of 10 per cent virus-brain. It was not recovered from blood similarly pooled from 4 mice sacrificed on the first, second, third and sixth days after intracerebral inoculation. These findings are characteristic of a highly neurotropic virus.

PATHOGENICITY IN OTHER ANIMALS

Limited experimentation was devoted to attempts to find another susceptible experimental animal beside the mouse. Six species of common laboratory animals were inoculated. All remained asymptomatic as may be observed in Table 2. All animals were adults with the exception of the chicks which were one-day old. Most of the tests were performed with early passage virus of relatively low titer. Koprowski and Hughes (1946) observed paralysis on the 12th day after intracerebral injection and death on the 13th in one of two rhesus monkeys inoculated with the original Ilhéus virus. The other rhesus remained well. Kerr (1952) obtained fatal encephalitis in 6 of 32 hamsters and 1 of 2 guinea pigs injected intracerebrally with 24th passage virus of the Brazilian strain. Evidently there is con-

TABLE 2

Susceptibility of common laboratory animals to the Honduran strain of Ilhéus virus

Animal Species	Number Inoculated	Route of Inoculation	Number of Passages of Inoculum	Result
White rabbit	2	Intracerebral	2	No illness
Guinea-pig	2	Intracerebral	2	No illness
Guinea-pig	2	Subcutaneous	2	No illness
Rhesus monkey	1	Intracerebral	2	No illness
Rhesus monkey	1	Subcutaneous	2	No illness
Chick	2	Intracerebral	11	No illness
Hamster, albino	2	Intracerebral	21	No illness
White rat	2	Intracerebral	24	No illness

siderable individual variability among animals of the same species in their reactions to this virus, the mouse being the only species so far studied to show highly uniform susceptibility.

FILTRABILITY AND PRESERVATION

The Honduran strain of Ilhéus virus like the Brazilian readily passes Seitz EK discs and Berkefeld "N" and "V" filters in 10 per cent suspension in normal monkey or rabbit serum-saline. Virus remained viable in mouse brain after 3 weeks storage in 50 per cent neutral glycerine-saline solution but not after 56 days. Koprowski and Hughes (1946) found a marked drop in the titer of their virus after storage for 41 days in 50 per cent glycerine solution. Virus remained alive and virulent in the mosquitoes after more than 7 months storage in dry ice. It also survived at least 20 days in a suspension of these mosquitoes in normal rhesus serum-saline in a commercial deep-freeze at approximately −30°C. It survived at least 3 months when desiccated in vacuo without appreciable loss of titer.

IMMUNOLOGICAL PROPERTIES

As previously stated yellow fever immune serums failed to protect mice against infection by the virus isolated from *Psorophora* in Honduras and vice versa. Similarly mice immunized against the Honduran strain failed to resist challenge inoculation with yellow fever virus and vice versa. Animal pathogenicity suggested a neurotropic virus of Group B according to the classification of Casals (1954). Using a mouse hyper-immune serum prepared here against the Honduran virus and forwarded to his laboratory, Dr. Casals obtained high titers in hemagglutination inhibition and complement-fixation tests against Group B antigens, permitting preliminary classification of the virus in this group. There was no reaction with Group A antigens. In the hemagglutination-inhibition reaction he reported a titer of 1-5120 or higher against a known Ilhéus antigen and titers of of 1-1280 against St. Louis, Japanese B and West Nile viruses. These viruses are very similar in pathogenicity and other properties. Smithburn (1952) in cross-neutralization tests first observed an immunological relationship among them. Considerable cross-reaction occurs in hemagglutination-inhibition and complement-fixation tests. However, titers with homologous antibody are significantly higher. By means of block complement-fixation tests in which serial dilutions of the serum were titrated against serial dilutions of the antigen, Dr. Casals obtained a reaction pattern against Ilhéus antigen practically identical to that of a known Ilhéus hyper-immune mouse serum.

DISCUSSION

The virus isolated from mosquitoes of the genus *Psorophora* captured in Honduras corresponds closely in animal pathogenicity, antigenic and other properties to that isolated originally by Laemmert and Hughes (1947). These authors recovered their strain from a mixed pool of *Aedes* and *Psorophora*. Furthermore, they showed that *Psorophora ferox*, as well as *Aedes aegypti* and *A. serratus*, are

able to transmit the virus from infant mouse to infant mouse. One of the Trinidad strains also was isolated from *Psorophora* and the other from a pool of eight genera including two species of *Psorophora*. The bulk of evidence so far accumulated, therefore, seems to incriminate *Psorophora*, especially *P. ferox*, most strongly in the epidemiology of the infection.

Ilhéus virus has been implicated as a human pathogen by the detection of antibodies in human serum both in Brazil by Laemmert and Hughes (1947) and in Trinidad by Downs, Anderson and Theiler (1956). Experimental infection in man was described by Southam and Moore (1951). They inoculated 19 human volunteers in advanced neoplastic disease, one of whom developed encephalitis and eight of whom had circulating virus. The isolation of Ilhéus virus in three such widely separated areas as Brazil, Trinidad and Honduras suggests the virus may be amply distributed throughout Central and South America.

CONCLUSIONS

Isolation of the virus of Ilhéus encephalitis from mosquitoes of the genus *Psorophora* captured on the north coast of Honduras is reported. This represents the first isolation of Ilhéus virus in Central America.

REFERENCE

Anderson, C. R., Aitken, T. H. G. and Downs, W. G., 1956. The isolation of Ilhéus virus from wild caught forest mosquitoes in Trinidad, *Am. J. Trop. Med. & Hyg.* **5**: 621–625.

Casals, J. and Brown, L., 1954. Hemagglutination with arthropod-borne viruses, *J. Exper. Med.* **99**: 429–449.

Downs, W. G., Anderson, C. R. and Theiler, M., 1956. Neutralizing antibodies against certain viruses in the sera of residents of Trinidad, B.W.I., *Am. J. Trop. Med. & Hyg.* **5**: 626–641.

Kerr, J. A., 1952. Studies on certain viruses isolated in the tropics of Africa and South America. Immunological reactions as determined by cross-complement-fixation tests, *J. Immunol.* **68**: 461–472.

Koprowski, H. and Hughes, T. P., 1946. The virus of Ilhéus encephalitis. Physical properties, pathogenicity and cultivation, *J. Immunol.* **54**: 371–385.

Laemmert, H. W. and Hughes, T. P., 1947. The virus of Ilhéus encephalitis. Isolation, serological specificity and transmission, *J. Immunol.* **55**: 61–67.

Pan American Sanitary Bureau, 1955. Yellow Fever Conference, *Am. J. Trop. Med. & Hyg.* **4**: 571–661.

Rodaniche, E. de, 1956. Survey of mosquitoes captured in Honduras for yellow fever virus, *Am. J. Trop. Med. & Hyg.* **5**: 480–482.

Smithburn, K. C., 1952. Studies on certain viruses isolated in the tropics of Africa and South America. Immunological reactions as determined by cross-neutralization tests, *J. Immunol.* **68**: 441–460.

Southam, C., and Moore, A. E., 1951. West Nile, Ilhéus and Bunyamwera virus infections in man, *Am. J. Trop. Med. & Hyg.* **31**: 724–741.

Trapido, H., and Galindo, P., 1955. The investigation of a sylvan yellow fever epizootic on the north coast of Honduras, 1954, *Am. J. Trop. Med. & Hyg.* **4**: 665–674.

TRANSMISSION OF EASTERN EQUINE ENCEPHALITIS TO HORSES BY *AEDES SOLLICITANS* MOSQUITOES

W. D. SUDIA, D. D. STAMM, R. W. CHAMBERLAIN AND R. E. KISSLING

Communicable Disease Center, Public Health Service, P.O. Box 61, Montgomery, Ala.

Although horses are highly susceptible to Eastern equine encephalitis (EEE), they appear rarely to develop enough virus in their blood to serve as an efficient source of infection for mosquitoes (Kissling *et al.*, 1954). On that basis it was assumed that the horse usually is only a dead end in the natural cycle of EEE infection and does little to help perpetuate the disease. On the other hand it was conceded that in the event of a high population of horse-feeding mosquitoes with low thresholds of infection, the horse might enter into the infection chain within the range of distribution of such selected mosquito species.

Studies on the EEE infection thresholds of many different species of mosquitoes (Chamberlain *et al.*, 1954; Schaeffer and Arnold, 1954) have indicated *Aedes sollicitans* to be one of the most susceptible, with a probable infection threshold of around $10^{-3.0}$[1]. Furthermore, TenBroeck and his associates (1935) succeeded in transmitting EEE infection from horse to horse with this species of mosquito. It appeared worthwhile, therefore, to perform quantitative studies with *Aedes sollicitans* to determine more accurately its capabilities in the spread of EEE infection among horses.

MATERIALS AND METHODS

The EEE virus used was a strain isolated from a mosquito, *Culiseta melanura* by Chamberlain and others (1951) and had been given 2 mouse-brain passages and 1 chick embryo passage. The 2 horses used were obtained in the vicinity of Montgomery, Ala. They were both in good condition and contained no detectable antibody to either EEE or Western equine encephalitis in their sera at the beginning of the experiment. Horse inoculations with virus were given either subcutaneously in the neck region with 0.03 ml. volume or by the bite of infected mosquitoes on a shaved area near the hip.

The *Aedes sollicitans* mosquitoes employed in this study were supplied as larvae by Mr. E. L. Seabrook, Director, Palm Beach County Anti-Mosquito Control District, West Palm Beach, Florida. They were shipped by air to the Virus Laboratory at Montgomery, Ala., where they were reared to adults and used in experiments when 2 to 7 days old. Except for blood meals given to infect the mosquitoes or to permit them to transmit infection, their only food consisted of soaked seedless raisins. All mosquito incubations were at 80°F and about 75 per cent relative humidity.

The experiment consisted of a) inoculating a normal horse (No. 1, a small 2-year-old mare) with EEE virus, b) taking rectal temperatures several times

[1] The infection threshold is defined as the lowest titer of virus in the blood capable of causing an infection in approximately 1 to 5 per cent of the mosquito specimens ingesting it.

daily, concurrent with the drawing of blood for virus titration, c) feeding groups of normal *A. sollicitans* upon the horse when its temperature showed a rise (evidence that the virus was most likely in high level in the blood), and d) refeeding some of these mosquitoes 2 weeks later upon a second normal horse (No. 2, a 4-year-old roan mare). Temperatures and blood samples were likewise taken on the second horse and normal mosquitoes fed upon it when viremia was most likely to be present. Determinations of infection rates and chick transmission rates of the various groups of mosquitoes, as well as quantitative estimations of the amounts of virus contained in individual mosquitoes, were made as previously described by Chamberlain *et al.* (1954) and Chamberlain, Corristan and Sikes (1954).

Titrations of virus were performed by intracerebral inoculation into 3-week-old CFW mice. The LD_{50} endpoints were calculated by the method of Reed and Muench (1938). In calculating the number of mouse intracerebral LD_{50} units of virus ingested by mosquitoes, the volume of blood taken per mosquito during feeding was considered to be 0.003 ml., or $\frac{1}{10}$ of a standard mouse intracerebral inoculation volume.

EXPERIMENTAL

Horse No. 1 was inoculated with 25,000 mouse intracerebral LD_{50} units of EEE virus. The temperature and viremia records on this horse following inoculation are given in Figure 1. Groups of approximately 50 to 80 normal *A. sollicitans* females were fed upon this horse at different times when, on the basis of fever data, the viremia was believed to be at high levels. The groups of mosquitoes, times of feeding, and the virus titers of the blood during the time of feeding were as follows:

Mosquito group	Hr. of mosq. feeding after inoculation of horse	Titer of virus in blood ingested
A	33–34	$10^{-4.4}$
B	36–37	$10^{-4.8}$
C	38–39	$10^{-4.8}$
D	40–41	$10^{-5.2}$
E	47–48	$10^{-4.4}$ to $10^{-4.8}$
F	48–49	$10^{-4.4}$ to $10^{-4.8}$

After 11 to 12 days' incubation, 10 mosquitoes from each feeding group were ground individually and inoculated into mice to approximate the infection rates in the various groups. As the strain of EEE virus used acts very quickly in mice, the results were known with a fair degree of certainty 2 days later, permitting estimation of infection rates. The percentages thus estimated of the mosquitoes infected in each group were: 10 for group A, 10 to 20 for group B, 20 for group C, 40 to 50 for group D, 40 to 50 for group E, and 20 for group F. On the strength of these findings some of the remaining group D mosquitoes were selected for refeeding on the 14th day of incubation upon normal horse No. 2. Estimating a

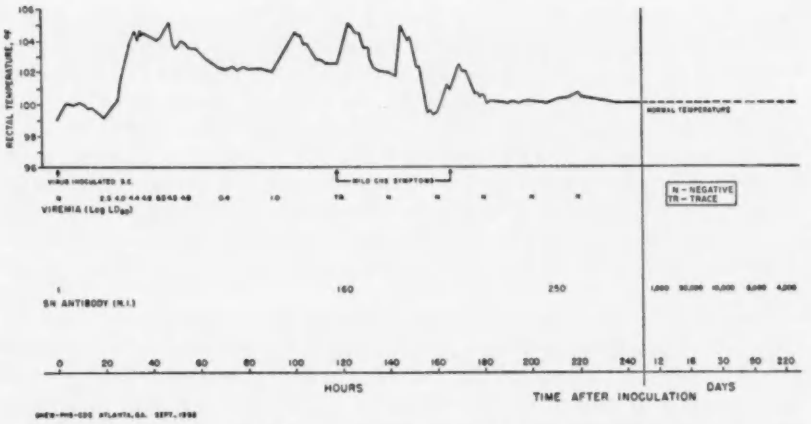


FIG. 1. Rectal temperatures, blood virus levels, and antibody response of horse No. 1 following subcutaneous inoculation with 25,000 mouse LD_{50} of EEE virus.

50 per cent infection rate for the group D specimens and a 50 per cent chick transmission rate for those infected, it was decided to refeed approximately 30 of these mosquitoes upon horse No. 2, a number probably including about 5 to 7 transmitters. A total of 29 actually fed.

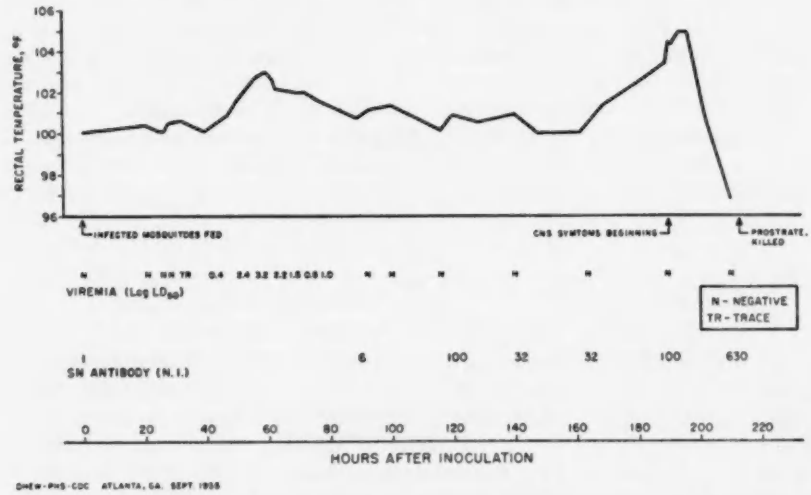


FIG. 2. Rectal temperatures, blood virus levels, and antibody response of horse No. 2 after being fed upon by EEE-infected *Aedes sollicitans* mosquitoes. (These mosquitoes had received their infection from horse No. 1)

TABLE 1

Infection of Aedes sollicitans *with EEE from a horse, transmission of EEE from horse to horse and horse to chicks by bite, and evidence of virus multiplication in mosquitoes*

Virus Source	Blood Virus Titer ($1/Log_{10}$)	Mouse i.c. LD_{50} Ingested per Mosq. (Log_{10})	Mosq. Group No.	No. Mosq. Becoming Infected/ No. Mosq. Feeding	Percentage Infected	Transmission (animal)	No. Mosq. Transmitting/ No. Mosq. Refeeding	Percentage Transmitting	Mouse i.c. LD_{50} Recovered per Mosq. (Log_{10})	Maximum Fold-increase of Virus in Mosq.
Horse No. 1	4.4	3.4	A	7/36	19	−(chicks)	0/25	0	3.5–5.0	40
	4.8	3.8	B	12/56	21	+(")	2/25	8	4.5–5.5	50
	4.8	3.8	C	8/57	14	+(")	2/25	8	4.1–6.5	500
	5.2	4.2	D	12/29	41	+(horse #2)	4/29?*	15?*	1.2–6.8	400
				6/23	26	+(chicks)	2/13	15	4.8–6.3	125
	4.5	3.5	E	14/48	29	+(")	1/25	4	3.0–6.0	300
	4.8	3.8	F	19/59	32	+(")	4/25	16	4.0–6.5	500
Horse No. 2	1.2	0.2	AA	0/64	0	−(")	0/25	0	0	0
	2.2	1.2	BB	0/76	0	−(")	0/24	0	0	0
	1.5 to 2.2	0.5 to 1.2	CC	0/58	0	−(")	0/24	0	0	0
	1.0	0.0	DD	0/72	0	−(")	0/25	0	0	0

* Estimated value—see text.

The temperature and blood virus pictures of horse No. 2 were followed closely, as shown in Figure 2. The 29 mosquitoes which had fed on horse No. 2 were tested immediately after the feeding by mouse brain inoculation for evidence of infection. Those specimens found to be positive were later titrated to determine the amount of virus each contained.

Twenty-three mosquitoes remaining in the group D series were given an opportunity to refeed upon ½-day-old chicks as a basis for estimating the transmission rate for that infected mosquito pool. A total of 13 were induced to feed, 2 of which transmitted. The remainder were subsequently ground and inoculated individually into mice, along with the 13 feeders, to determine the infection rate. On the 17th to 18th day of incubation random samples of 25 mosquitoes each from groups A, B, C, E, and F were tested similarly to determine their transmission and infection rates.

Normal *A. sollicitans* were held in readiness to feed upon horse No. 2 in case it showed evidence of infection, for it was desired to continue the study by attempting infection of the mosquitoes from a horse which had received its infection from mosquitoes. On the 60th hour after the infected group D mosquito feeding, the horse showed a significant temperature rise. Feeding of groups of 64 to 72 normal mosquitoes each was commenced at that time. The times of feeding and the virus titers of the blood ingested were as follows:

Mosquito group	Hr. of mosquito feeding after exposure of horse to infection	Titer of virus in blood ingested
AA	60–61	$10^{-1.2}$
BB	62–63	$10^{-2.2}$
CC	63–64	$10^{-1.5}$
DD	75–76	$10^{-1.9}$

These mosquitoes were then incubated for two weeks before testing. It was found that none had become infected.

The pertinent experimental findings of the entire experiment are summarized in Table 1. In it are shown infection of *A. sollicitans* with EEE from a horse, transmission of EEE from horse to horse and horse to chick by mosquito bite, and evidence of virus multiplication in mosquito tissue. Data on development of neutralizing antibody by horses Nos. 1 and 2 are included in Figures 1 and 2.

DISCUSSION

On the basis of past experimentation with this strain of EEE virus in horses by Kissling and others (1954), the viremia of horse No. 1 was exceptionally high. A titer of $10^{-4.0}$ occurred as early as the 26th hour following inoculation, and titers of $10^{-4.4}$ or higher were reached from about the 30th hour to more than 50 hours. The highest titer attained was $10^{-5.5}$, at least 2 logs beyond that expected in most inoculated horses. Virus in trace amounts persisted until at least the 117th hour. In all, virus was present in the blood in detectable amounts for about 100 hours. In spite of the high viremia and rapid onset, however, the horse recovered with no apparent sequelae.

As shown in Table 1, 14 to 41 per cent of the normal *A. sollicitans* mosquitoes which fed in several groups upon this horse when its blood virus titered $10^{-4.4}$ to $10^{-5.2}$ became infected. Chick transmission rates determined for these various mosquito groups ranged from 0 per cent for those which had fed upon the $10^{-4.4}$ titered blood to 15 and 16 per cent for those which had fed upon the $10^{-5.2}$ and $10^{-4.8}$ titered blood. Since a chick transmission rate of 15 per cent was determined for group D mosquitoes (the group supplying the 29 specimens which successfully transmitted EEE infection to normal horse No. 2 by bite), it can be estimated that approximately 4 of the 29 (15 per cent) transmitted virus to this horse. It is possible, however, that more than 4 transmitters may have been present in this feeding group, as 41 per cent of the 29 were infected, compared to only 26 per cent of the other specimens from the same pool used to set the transmission rate. If it is assumed that the ratio of transmitters to infected specimens remained constant in the two series, as many as 7 of the mosquitoes feeding on horse No. 2 may have transmitted measurable doses of virus.

Also shown in Table 1 is evidence of multiplication of EEE virus in the tissues of mosquitoes. Up to 500 times as much virus as the amount ingested was recovered from some of the specimens.

The blood virus picture of horse No. 2 is shown in Figure 2. A trace of virus was detected in the blood 33 hours following the infected group D mosquito

feeding. This increased to a titer of $10^{-3.2}$ at 53–55 hours, then declined. The last virus was detected at 77 hours. In all virus was present in the blood for a period of about 48 hours, but in a concentration possibly infective for mosquitoes for less than 4 hours. In spite of the small amount of virus in the blood, however, the infection was fatal.

The temperature peak of horse No. 2 came a few hours after the viremia peak, probably due to the fact that the maximum viremia was of such short duration. The times for feeding normal mosquitoes in attempted infection from this horse were selected upon the basis of the temperature rise. Therefore, the feedings were not at the time the virus in the blood titered $10^{-3.2}$ (about threshold level for *A. sollicitans*), but only $10^{-1.0}$ to $10^{-2.2}$, all below threshold level. Consequently, none of 270 specimens ingesting the blood containing virus in these titers became infected.

In consideration of the results of past EEE threshold studies with several species of mosquitoes (Chamberlain *et al.*, 1954), it was to be expected that horse-to-horse transmissions of EEE infection were possible, although uncommon. Although the usual concentrations of virus in the blood of experimentally infected horses were below mosquito-infecting levels, an occasional one had titers of around $10^{-4.0}$. Feeding upon virus at this level might induce mosquito infections, especially among species such as *A. sollicitans* with relatively low infection thresholds. Horse No. 1 in the present experiment possessed a higher viremia ($10^{-5.5}$) than any of over 20 horses previously tested in this laboratory. Virus in this titer was adequate for infecting approximately half of the *A. sollicitans* ingesting it. It would also have been adequate for infecting some other species of mosquitoes, such as *Aedes aegypti* and *A. triseriatus*.

The significance, in an epizootic, of even an occasional horse being infective for mosquitoes cannot be overlooked. In the presence of a large number of low-threshold mosquitoes and a high percentage of nonimmunes in the horse and bird populations, such a horse may add momentum to a flood of infection. In view of the low mosquito-infecting potential of the horses compared with the high potential of birds, however, it would appear necessary to have many infected horses in evidence before this method of epizootic spread assumes much importance.

Human infection resulting from the feeding of horse-infected mosquitoes must also be considered a possibility. However, in view of past epizootics and our present knowledge of the magnitude of the infections in birds, it is not likely that human infections stemming from horses occur with a degree of frequency sufficient to cause concern. In the 1947 southwest Louisiana outbreak, which caused deaths in more than 10,000 horses (and probably lighter infections in 5 to 10 times that many), only 8 human cases were reported. While one may suspect that these 8 received their infections from horse-infected mosquitoes, it is possible that bird-infected mosquitoes were responsible instead. In 1949, an outbreak killing several hundred horses in north central Louisiana occurred, with no human cases reported.

Vaccination of horses to protect them from infection and from assuming a

possible role in virus propagation appears to be an obvious recommendation. It is a matter of record, however, that in more highly developed agricultural communities in this country the horse population is greatly reduced. In more undeveloped and primitive areas of the EEE range, where horses may be present in large numbers, they are often not considered of sufficient value to warrant the expense of vaccination. Moreover, the pasture lands in these areas are usually such as to permit close association of horses, birds, and mosquitoes, and it is not necessary to hypothesize horse-to-horse transmission to account for high infection in horses. In these areas it seems possible that mosquitoes infected from birds could account for most of the horse infections as well as the infrequent human cases of EEE which occur.

SUMMARY

Aedes sollicitans mosquitoes became infected with Eastern equine encephalitis (EEE) from feeding upon an inoculated horse. These mosquitoes, after two weeks' incubation, transmitted infection by bite to a normal horse. Attempts to infect more mosquitoes by permitting them to feed upon this second horse met with failure. The virus which circulated in the blood of the inoculated horse titered as high as $10^{-5.5}$, a level not commonly attained in horses. The horse infected by mosquito bite had a lower, more usual concentration of virus in its blood, inadequate to infect mosquitoes. The results of these studies prove it possible for an occasional horse to serve as an EEE infection source for mosquitoes. It is believed, however, that horses rarely play an important role in EEE propagation.

REFERENCES

CHAMBERLAIN, R. W., CORRISTAN, E. C., AND SIKES, R. K., 1954. Studies on the North American arthropod-borne encephalitides. V. The extrinsic incubation of eastern and western equine encephalitis in mosquitoes, *Am. J. Hyg.* **60**: 269–277.

CHAMBERLAIN, R. W., RUBIN, H., KISSLING, R. E., AND EIDSON, M. E., 1951. Recovery of virus of eastern equine encephalomyelitis from a mosquito, *Culiseta melanura* (Coquillett), *Proc. Soc. Exp. Biol. & Med.* **77**: 396–397.

CHAMBERLAIN, R. W., SIKES, R. K., NELSON, D. B., AND SUDIA, W. D., 1954. Studies on the North American arthropod-borne encephalitides. VI. Quantitative determinations of virus-vector relationships, *Am. J. Hyg.* **60**: 278–285.

KISSLING, R. E., CHAMBERLAIN, R. W., EIDSON, M. E., SIKES, R. K., AND BUCCA, M. A., 1954. Studies on the North American arthropod-borne encephalitides. II. Eastern equine encephalitis in horses, *Am. J. Hyg.* **60**: 237–250.

REED, L. J., AND MUENCH, H., 1938. A simple method of estimating fifty per cent endpoints, *Am. J. Hyg.* **27**: 493–497.

SCHAEFFER, M., AND ARNOLD, E. H., 1954. Studies on the North American arthropod-borne encephalitides. I. Introduction. Contributions of newer field-laboratory approaches, *Am. J. Hyg.* **60**: 231–236.

TENBROECK, C., AND MERRILL, M. H., 1935. Transmission of equine encephalomyelitis by mosquitoes, *Am. J. Path.* **11**: 847.

STUDIES OF DENGUE FEVER VIRUS (HAWAII MOUSE ADAPTED) IN LACTATING HAMSTERS

REGINALD L. REAGAN, FRANCES S. YANCEY AND A. L. BRUECKNER

Virus Laboratory, Live Stock Sanitary Service, University of Maryland, College Park, Maryland

The dengue fever virus strain (Hawaii mouse adapted) used for this study was secured from the American Type Culture Collection of Washington, D. C. This strain was furnished to that group by Dr. A. B. Sabin of Children's Hospital, Cincinnati, Ohio. Following its isolation in 1944, the virus was maintained for several serial passages in human volunteers and was propagated in mice by intracerebral passage Sabin *et al.*, (1945). Lyophilized mouse brain of the 114th intracerebral mouse passage was received at this Maryland laboratory to initiate the current study. In its present form the virus is highly pathogenic when injected intracerebrally into mice but is not pathogenic for guinea pigs, rabbits or cotton rats, as reported by Sabin (1945). Mice exhibited symptoms of central nervous system involvement 5 to 6 days after inoculation of 0.03 cc. of a 10 per cent suspension.

EXPERIMENTAL METHODS AND RESULTS

The following study was undertaken to see if this neurotropic virus could be transmitted in the mother's milk to sucklings. Seven lactating hamsters and their sucklings were placed in individual metal boxes with bedding. The sucklings were about 12 days old, and as they are usually weaned at 16 to 18 days, they were removed from the parent 6 days after her inoculation with the respective virus. The lactating hamster from Group 1 to 6 was given 0.2 ml. of the above described dengue fever bearing mouse brain suspension via the intraperitoneal cavity. The number 7 group (control) was inoculated with normal mouse brain suspension. All areas of injection were sterilized with iodine for one hour before the parent from each of the seven groups were placed with her sucklings. During the 6 days following inoculation of the mother, the groups were not disturbed. Dog checkers were fed to each of the 7 groups daily, which had access to a large bottle of water for drinking. At the end of the sixth day, the sucklings from each group were weaned and placed in special glass jars. None of the lactating adults (6 groups + control), developed symptoms of nervous system involvement found in animals exposed to this virus after intracerebral inoculation. They were sacrificed after a 21-day observation period, their brains were removed aseptically, ground in a mortar with alundum and diluted to a 20 per cent suspension with physiological saline and injected intracerebrally into 3 week old Swiss albino mice. The mice appeared normal and were discarded after a 21 day observation period. The six groups of weaned hamsters from the six infected lactating animals and the control hamster were fed, watered and checked twice daily (Table 1) for nervous symptoms. Four hamsters from groups 1, 2, and 4

TABLE 1

Response of suckling hamsters after weaning from lactating parent to dengue fever virus (Hawaii mouse adapted)

Group #	Suckling #	Onset of disease from day of mothers exposure to the virus	Neutralization test
1	1	12–15 days	Negative
	2		Positive
	3		Negative
	4		Negative
	5		Negative
2	1		Negative
	2	10–12 days	Positive
	3		Negative
	4		Negative
3	1		Negative
	2		Negative
	3		Negative
	4		Negative
	5		Negative
	6		Negative
4	1		Negative
	2	15–18 days	Positive
	3		Negative
	4	12–14 days	Positive
	5		Negative
	6		Negative
5	1		Negative
	2		Negative
	3		Negative
	4		Negative
	5		Negative
6	1		Negative
	2		Negative
	3		Negative
	4		Negative
	5		Negative
	6		Negative
7	1		Negative
	2		Negative
Control	3		Negative
	4		Negative
	5		Negative
	6		Negative

showed symptoms of nervous system involvement. Brains from all suckling hamsters showing nervous symptoms were removed aseptically, pooled and ground in a mortar with physiological saline to a 20 per cent suspension and neutralization studies were conducted in Swiss albino mice using dengue rabbit antiserum (in order to confirm that dengue virus was present in the suckling hamster brain).

At the end of 21 days all suckling hamsters that had no symptoms of nervous

system involvement were sacrificed and their brains removed aseptically and pooled, as were also the control group #7. All brains were ground in a mortar with alundum and diluted to a 20 per cent suspension with physiological saline. The two pooled groups were injected intracerebrally into Swiss albino mice. The mice showed no signs of central nervous system involvement after a 21 day observation period and were discarded.

SUMMARY

From this observation it appears that dengue fever virus (Hawaii mouse adapted) may be transmitted from lactating hamsters to suckling hamsters. None of the mothers showed symptoms of nervous system involvement over a 21 day observation period. Mouse tests of their brains were negative. Four out of 32 suckling hamsters developed symptoms of nervous system involvement and their brains were shown to contain dengue fever virus by neutralization tests conducted intracerebrally in Swiss albino mice. The control sucklings and sucklings from the exposed mothers showing no signs of central nervous system involvement were sacrificed and their brains tested by the intracerebral mouse test, but no virus was demonstrated.

REFERENCE

SABIN, A. B., AND SCHLESINGER, R. W., 1945. Production of immunity to dengue with virus modified by propagation in mice, *Science* **101**: 640–642.

STUDIES IN SHIGELLOSIS

VI. Observations on Incidence and Etiology of Diarrheal Disease in Egyptian Adults.[1]

THOMAS M. FLOYD[2], JAMES W. BLAGG AND M. A. KADER

United States Naval Medical Research Unit No. 3, Cairo, Egypt

The importance of diarrheal disease as a public health problem in Egypt, particularly as a cause of morbidity and mortality among infants and young children, has been considered in previous reports of studies in shigellosis in this country (Floyd, 1954; Higgins and Floyd, 1955; Higgins, Floyd and Kader, 1955). While these investigations, and others reviewed in the reports cited above, provided a considerable amount of information on the incidence and etiology of the disease in children, relatively little was known of similar aspects of gastrointestinal infection in Egyptian adults. Admission rates to government hospitals and clinical studies on hospitalized patients indicated that diarrheal disease was prevalent among the older age group and that the etiology was often the same as that found in children. However, data of this type were of limited application in establishing morbidity among adults in the general population since the majority of cases would not require hospitalization. Available information was also of little value in comparing incidence among adults and children living in the same environment, or in evaluating the relative importance of various etiological agents of the disease. Of particular importance to the present series of studies was the need of more precise data to determine the effects of years of constant exposure in a highly endemic environment on the incidence and severity of shigellosis in adults. The present study was designed to provide the required information through frequent and long-term bacteriological, parasitological and clinical observations on a group of adults living in a typical Egyptian village.

METHODS

Seventy adults, between 17 and 40 years of age, were selected for study. All were either housewives or farmers and lived in Sindbis, the village in which other investigations on diarrheal disease had been conducted. A description of the environment has been presented earlier (Higgins and Floyd, 1955). Selection, which was made through interviews by the medical officer of the Rural Health Center, was influenced by an effort to equalize the group between the sexes and by an attempt to obtain approximately equal numbers in five-year age groups between 15 and 40.

On admission to study, all subjects were examined by the medical officer and

[1] The opinions or assertions contained herein are the private ones of the authors and are not to be construed as official or reflecting the views of the Navy Department or the naval service at large.

[2] Present address: Naval Medical Research Institute, National Naval Medical Center, Bethesda 14, Maryland.

a personal history taken. Blood counts, urinalyses, bacteriological and parasitological examinations of stool and urine specimens, blood cultures, Kahn tests, and *Brucella* and typhoid-paratyphoid antibody titer determinations were made. The adults were followed for a period of eight months, between January and August, 1954, by weekly visits of nurse-technician teams. During visits the general state of health of individuals was determined by questioning and observation. Temperatures were taken and the number and character of stools in the previous 24-hour period recorded. Rectal swab cultures and fresh stool specimens were obtained. Ill patients were referred to the Rural Health Center for diagnosis and treatment. Rectal swabs were cultured for the presence of enteric pathogens and stool specimens were examined for helminths and protozoa by methods described in the reports cited earlier.

For purposes of comparison a group of 93 children, between one and six years old, living in Sindbis, was followed in an identical manner with the exception that parasitological examinations were not made. Selection and methods employed with these subjects, the control group of earlier phases of this series of studies, have already been described (Higgins and Floyd, 1955; Floyd, 1956).

Definitions of diarrheal episodes, symptomatic and asymptomatic *Shigella* infections, and the factors affecting results were the same as those presented in the reports cited above.

RESULTS

There were 55 adults remaining at the end of the eight months' study. The mean age, among 24 males and 31 females, was 25.5 years. Fifteen were dropped from the study either for lack of cooperation or because they moved from the village. The group of children had diminished to 86 by the time observations were terminated. Six were removed because of failure of mothers to cooperate and one had died.

There were 66 episodes of diarrheal disease among the adults, or an average of 1.2 episodes per subject in eight months. The control group of children experienced 151 episodes, or an average of 1.7 attacks per child, during the same period. As shown in Table 1, the estimated annual attack rates per thousand were 1,800 and 2,600 cases respectively.

Frequency of diarrheal episodes in the two groups is presented in Table 2. Thirteen (23.6%) of the adults reported no attacks of diarrhea. Roughly two-

TABLE 1

Incidence of diarrheal disease in Egyptian adults and children

Subjects	Number	Diarrheal Disease		
		Observed episodes		Estimated annual attack rate/1000
		Number	Av/subj	
Adults	55	66	1.2	1800
Children	86	151	1.7	2600

TABLE 2

Frequency of diarrheal episodes in Egyptian adults and children

Number Episodes	Adults		Children	
	Number	Per cent	Number	Per cent
0	13	23.6	11	12.8
1	25	45.5	26	30.2
2	11	20.0	28	32.6
3	5	9.1	16	18.6
4	1	1.8	4	4.6
5	0	0.0	1	1.2
Total.....	55	100.0	86	100.0

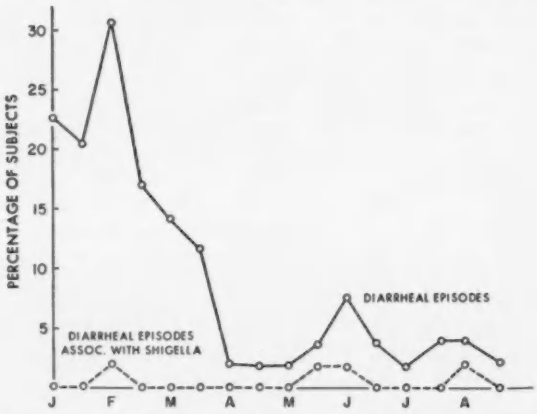


FIG. 1. Incidence of total diarrheal episodes and diarrheal episodes associated with Shigella in Egyptian adults.

thirds of the group experienced one or two attacks and only six had more than two episodes. In comparison, the spread among the group of children was more uniform. A smaller percentage had no episodes and a larger proportion experienced three or four episodes.

Seasonal incidence of total diarrhea and episodes associated with *Shigella* in the two groups, plotted at bi-weekly intervals, is shown in Figs. 1 and 2. During January, February and March all ages experienced diarrhea concurrent with an outbreak of upper respiratory infections. The majority, 49 or 74.2 per cent, of the diarrheal episodes observed among adults occurred during this period. From April until the middle of August there were only 17 attacks of diarrhea in this age group, or an average of 0.3 attacks per subject. Meanwhile 100 episodes, or an average of 1.1 per subject, were observed in the children during the latter period.

Five (7.5%) of the 66 episodes occurring in adults were associated with the

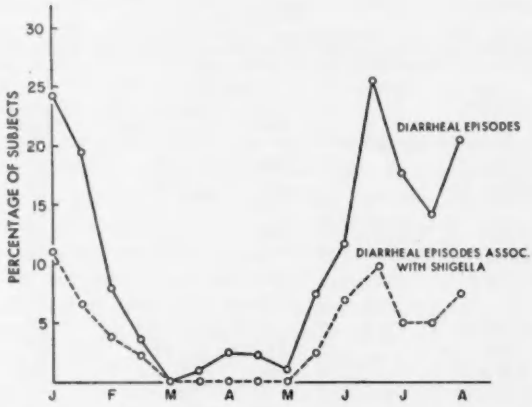


FIG. 2. Incidence of total diarrheal episodes and diarrheal episodes associated with Shigella in Egyptian children.

TABLE 3

Incidence of Shigella *and* Salmonella *in diarrheal episodes*

Subjects	Diarrheal Episodes						
	Total number	Shigella present		Salmonella present		Undetermined	
		Number	Per cent	Number	Per cent	Number	Per cent
Adults	66	5	7.5	1	1.5	60	91.0
Children	151	32	21.1	3	1.9	116	76.8

TABLE 4

Relationship of Shigella *infections and diarrheal disease*

Subjects	Shigella Infections				
	Total number	Diarrhea present		Diarrhea absent	
		Number	Per cent	Number	Per cent
Adults	38	5	13.1	33	86.9
Children	72	38	52.7	34	47.3

presence of *Shigella*. These organisms were recovered during 32 (21.1%) of the 151 attacks in children. There was one bacteriologically confirmed case of typhoid fever among adults and three in the group of children. Thus, six (9%) of the episodes of diarrhea in adults were attributed to either *Shigella* or *Salmonella* while the remaining 60 (91%) were of undetermined etiology. Thirty-five (23%) of the diarrheal attacks in children were either bacillary dysentery or typhoid fever and 116 (77%) were of undetermined etiology.

Shigella were recovered from 38 (2.5%) of 1,518 rectal swab cultures on

TABLE 5

Incidence of intestinal parasites in 1494 stools and cumulative percentages of infected subjects

Parasites	Positive Stools		Infected Subjects	
	Number	Per cent	Number	Per cent
Entamoeba histolytica	589	39.4	53	96.3
Iodamoeba butschlii	158	10.5	38	69.0
Giardia lamblia	97	6.4	31	56.3
Chilomastix mesnili	56	3.7	29	52.7
Ascaris lumbricoides	798	53.4	52	94.5
Hookworm	38	2.5	5	9.1
Trichostrongylus	66	4.4	29	52.7
Enterobius vermicularis	51	3.4	31	56.3
Schistosoma mansoni	775	51.8	50	90.9
Schistosoma hematobium	85	5.6	29	52.7

TABLE 6

Incidence of intestinal parasites in 66 diarrheal episodes in Egyptian adults

Parasites	Present		Absent	
	Number	Per cent	Number	Per cent
Entamoeba histolytica	42	63.6	24	36.4
Iodamoeba butschlii	18	27.3	48	72.7
Giardia lamblia	8	12.1	58	87.9
Chilomastix mesnili	11	16.7	55	83.3
Ascaris lumbricoides	48	72.7	18	27.3
Hookworm	6	9.1	60	90.9
Trichostrongylus	7	10.6	59	89.4
Enterobius vermicularis	7	10.6	59	89.4
Schistosoma mansoni	57	86.3	9	13.7
Schistosoma hematobium	22	33.3	44	66.7

TABLE 7

Relationship of intestinal parasites with diarrheal disease in Egyptian adults

Parasites	No. Pos. Stools	Diarrhea Present		Diarrhea Absent	
		Number	Per cent	Number	Per cent
Entamoeba histolytica	589	86	14.6	503	85.4
Iodamoeba butschlii	158	26	16.5	132	83.5
Giardia lamblia	97	11	11.3	86	88.7
Chilomastix mesnili	56	12	21.4	44	78.6
Acaris lumbricoides	798	133	16.7	665	83.3
Hookworm	38	6	15.8	32	84.2
Trichostrongylus	66	11	16.7	55	83.3
Enterobius vermicularis	51	8	15.6	43	84.3
Schistosoma mansoni	775	172	22.2	603	77.8
Schistosoma hematobium	85	29	34.1	56	65.9

adults and from 72 (2.9%) of 2,435 cultures on children. As shown in Table 4, five (13.1%) of the *Shigella* isolations from adults were made during episodes of diarrhea while 35 (86.9%) were obtained from asymptomatic subjects. Thirty-eight (52.7%) of the *Shigella* infections in children were associated with diarrhea and 34 (47.3%) were non-diarrheal.

During the eight months' study 1,494 stools were examined for the presence of intestinal parasites. In Table 5 are shown the percentages of specimens in which the various parasites were found and the cumulative percentages of infected subjects. More than one-half the stools contained eggs of *Schistosoma mansoni* and *Ascaris lumbricoides*. Trophozoites or cysts of *Entamoeba histolytica* were demonstrated in almost 40 per cent. More than 90 per cent of the adults were infected at least once with these three parasites. On the average, infected individuals had 11.1 positive stools of *E. histolytica*, 15.5 positive stools of *A. lumbricoides* and 15.5 positive stools of *S. mansoni*.

The incidence of intestinal parasites in the 66 episodes of diarrhea observed in adults is shown in Table 6. *Entamoeba histolytica* was present in 63.6 per cent, *A. lumbricoides* in 72.7 per cent and *S. mansoni* in 86.3 per cent of the episodes.

The relationship between positive specimens and the existence or absence of diarrheal symptoms is presented in Table 7. With the exception of *S. hematobium*, roughly 85 per cent of the positive specimens were obtained from subjects without diarrhea.

DISCUSSION

The results indicated that diarrheal disease was common among the Egyptian adults of this study. During the eight months of observation there was an average of more than one attack per subject. Projecting the observed data for 12 months resulted in an average of almost two episodes per person per annum. It is felt that this is a minimal estimate. From approximately 1,500 observations on individuals who considered themselves to be well at the time, it was noted that the average Egyptian village adult defecated two or more times a day. These stools often were semi-fluid or mushy in consistency. Considering this bowel habit, it is not unlikely that mild episodes of diarrhea were overlooked and the episodes that were reported represented the more serious attacks.

It was evident, however, that diarrheal disease was less prevalent in the older age group than among children living in the same environment. The estimated annual attack rate in the latter is also considered to be minimal, since the study did not include the fall months when a secondary period of high incidence was expected to occur in these subjects. By actual observation there were 299 episodes of diarrhea, or an average of 3.4 attacks per child and an annual attack rate of 3,400 cases per 1,000, in this same group of children during the preceding 12 months. This two to one ratio has been noted in other highly endemic areas (Watt and Hardy, 1945).

During the winter months both age groups were equally affected by an outbreak of upper respiratory tract infections which apparently had diarrheal manifestations. The etiology of these infections was not determined. Incidence

of diarrheal disease in adults during the summer months had little relation to that observed in children. In May, June and July, when children experienced the usual seasonal rise in diarrhea in general and in diarrhea associated with *Shigella*, the infection rate in adults was only one-fourth that of the younger age group.

Only a small proportion of the cases in adults were shown to be associated with *Shigella*. While these organisms were demonstrated in rectal swab cultures in about the same percentage in both groups, only 13 per cent of the infections could be related to diarrhea as compared with 52 per cent in children. From these results, and from data presented in a previous report (Floyd *et al.*, 1956), it would appear that susceptibility of individuals under constant exposure decreases with age.

Attempts to relate the presence of intestinal parasites with diarrheal disease in adults met with the same difficulties encountered in children living in this environment (Higgins *et al.*, 1955). While forms such as *E. histolytica*, *A lumbricoides* and *S. mansoni* were present in 60 to 80 per cent of the cases of diarrhea in adults, they were present also in a large proportion of stools from asymptomatic individuals. With the exception of the schistosomes, more than 80 per cent of all infections identified in this study were non-diarrheal. However, the importance of schistosomes, intestinal protozoa and *Ascaris* as incitants of diarrhea and in aggravating other intestinal disorders cannot be overlooked. In all probability they were of equal importance to *Shigella* in the etiology of diarrhea observed in this group of adults.

One can only speculate upon the role of viral agents, other bacteria and dietary factors in the production of diarrhea in this group of adults.

The occurrence of four cases of typhoid fever among 141 individuals was of interest. It was estimated that the annual attack rate of the disease in Egypt was in the neighborhood of 1,200–1,800 cases per 100,000 population (Weir *et al.*, 1952). The group of adults and children in this study could not be considered a representative sample, being heavily weighed with young susceptibles, but the observed cases were the equivalent of an attack rate of 4,200 per 100,000 which suggests that Weir and co-workers did not over-estimate.

SUMMARY

The incidence and etiology of diarrheal disease in a group of 55 Egyptian village adults were studied by weekly clinical, bacteriological and parasitological observations over a period of eight months. There were, on the average, 1.2 episodes of diarrhea per subject during this time, or an estimated annual attack rate of 1,800 cases per 1,000. *Shigella* or *Salmonella* were demonstrated in 9 per cent of the episodes. The subjects were heavily parasitized with *E. histolytica*, *A. lumbricoides* and *S. mansoni* but it was difficult to associate the presence of these parasites with the diarrheal episodes.

Adults were infected with *Shigella* at about the same frequency as children living in the same environment, but a far greater percentage of the infections in

adults were asymptomatic with respect to diarrhea. This suggests that, under constant exposure, susceptibility to infection with *Shigella* decreases with age.

REFERENCES

FLOYD, T. M., 1954. Incidence of *Shigella* infections in Egyptian village children, *Am. J. Trop. Med. & Hyg.* **3**: 294–301.

FLOYD, T. M., HIGGINS, A. R., AND KADER, M. A., 1956. Studies in shigellosis. V. The relationship of age to the incidence of *Shigella* infections in Egyptian children, with special reference to shigellosis in the newborn and in infants in the first six months of life, *Am. J. Trop. Med. & Hyg.* **5**: 119–130.

HIGGINS, A. R., AND FLOYD, T. M., 1955. Studies in shigellosis. I. General considerations, locale of studies and methods, *Am. J. Trop. Med. & Hyg.* **4**: 263–270.

HIGGINS, A. R., FLOYD, T. M., AND KADER, M. A., 1955. Studies in shigellosis. II. Observations on incidence and etiology of diarrheal disease in Egyptian village children, *Am. J. Trop. Med. & Hyg.* **4**: 271–280.

WATT, J., AND HARDY, A. V., 1945. Studies of the acute diarrheal diseases. XIII. Cultural surveys of normal population groups, *Pub. Health Rep.* **60**: 261–273.

WEIR, J. M., WASIF, I. M., HASAN, F. R., ATTIA, S. EL-D. M., AND KADER, M. A., 1952. An evaluation of health and sanitation in Egyptian villages, *J. Egyptian Pub. Health Assn.* **27**: 55–114.

BIOLOGICAL AND BACTERIOLOGICAL STUDIES ON *ESCHERICHIA COLI* IN THE HOUSEFLY, *MUSCA DOMESTICA*

ROBERT L. INGRAM[1], JOSEPH R. LARSEN, JR.[2] AND WARREN F. PIPPEN[3]

Medical Laboratories, Army Chemical Center, Maryland and Camp Detrick, Frederick, Maryland

The common housefly, long a companion of man and incriminated as a vector (both mechanical and biological) of disease-producing organisms, has, more specifically, been associated closely with the enteric diseases. In this connection, it has even been referred to as "the most dangerous insect known" (West, 1951, p. 264) though the literature gives little precise information about its capability as a vector. Thus, although there have been several recent laboratory studies of the ability of the fly to transmit certain organisms (Hawley *et al.*, 1951; Melnick and Penner, 1952; and others), there are still differences of opinion as to the role of the fly in the epidemiology of the various diseases with which it has been associated. Further investigations of the transmission of enteric bacteria by flies are obviously needed.

The present study was designed to test certain techniques for the laboratory study of such problems, and to provide information on the ability of flies to take up and transmit *Escherichia coli* under laboratory conditions. Information was sought on the following points.

1. The effect of kind of food, frequency of feeding, and temperature on survival of immobilized flies.
2. The mortality of caged flies under laboratory conditions.
3. The fate of *E. coli* ingested by immobilized flies.
4. The effect of variation in food on the propagation of ingested bacteria.
5. The feasibility of using radioactively labeled organisms for studying bacterial dissemination by flies.
6. The rate of uptake of *E. coli* from an infected source by the fly.

MATERIALS AND METHODS

All of the flies used in these studies were reared on horsemeat, as described by Snyder (1949), in an insectary maintained at 90°F. and 35 per cent relative humidity. The flies were isolated within a two-hour period after emerging. Those to be used for studying the fate of organisms passed through the fly were starved for 24 hours, mounted on paraffin-coated glass rods and fed the bacteria suspended in a 1 M sucrose-skimmed milk solution. The flies were fed the desired number of organisms from a calibrated micrometer-driven 0.25 ml. tuberculin syringe. Afterwards the flies were fed the sucrose-milk solution once a day.

[1] Present address, Camp Detrick, Frederick, Maryland.

[2] Present address, Department of Pathobiology, School of Hygiene and Public Health, John Hopkins University, Baltimore, Maryland.

[3] Present address, Ramey Air Force Base, Puerto Rico.

Except for handling, the flies were kept in an insectary maintained at 95°F. and 35 per cent relative humidity or at 70°F. and 70 per cent relative humidity.

A strain of *E. coli*, resistant to 1 gm. streptomycin/100 ml. of medium, was used throughout these studies. The organisms for the individual fly feedings were grown in nutrient broth for 18 hours at 37°C. Vials containing 5 ml. of normal saline were placed under the flies for recovering the organism; aliquots were taken from the vials for making pour-plates of nutrient agar to which had been added 1 gm. streptomycin/100 ml. The plates were incubated for 20 to 24 hours at 37°C.

Bacteria were tagged by growing them in nutrient broth which contained approximately 2½ millicuries of P^{32} per ml. The inoculated tubes were rotated in the incubator at about 10 revolutions per minute and incubated for 18 hours at 37°C. The bacteria were collected from the flies and plated as previously described. One ml. from the recovery vial was put in an aluminum foil planchet and evaporated to dryness under an infra-red lamp. Radioactivity counts were made with a Berkley Decimal Scaler, Model 2000, with a Tracer Lab PGC-1B83GM tube. The thickness of the Geiger tube window was 1.29 mg. per sq. cm.; all samples were measured 1 cm. from the window. Counts were corrected for background, coincidental counting, absorber correction when absorbers were used, and decay. The efficiency of the tube was checked with two known standards (Pb^{210} and Ux^{234}).

Periodic checks were made on the accuracy of the techniques in this investigation. Plates were counted twice by the same individual and by two different individuals. Direct plating was done to determine whether we were delivering the theoretical number of organisms from the feeding apparatus. Dilutions of stock P^{32} samples from Oak Ridge were evaporated and counted. The results were checked against a time-decay chart showing the amount of radioactivity in the samples. In all cases the per cent error was small and no corrections were necessary.

RESULTS

The effect of different conditions on the survival of the fly. Before we could begin to determine the efficiency of this insect as a disseminator of *E. coli* it was necessary to determine the best conditions for the survival of the flies under our experimental design. The kind of food, frequency of feeding, temperature, and watering were believed to be of importance. The flies were mounted on glass rods, and 10 flies were used for each condition. Three foods were used: milk, sucrose-milk, and Brain-Heart Infusion. The frequencies of feeding were either 2 or 3 times a day. Some groups of flies were offered water, others not. These conditions were set up in a 3 x 2 x 2 factorial design and the log mean ST (survival time) of the 12 sets of conditions was tested for significant differences by analysis of variance. The mortality rate (standard deviation) for the 12 sets of conditions was tested for homogeneity by means of Bartlett's test.

Although the data proved not to be homogeneous in each of the 12 treatments, the analysis of variance indicates that milk and sucrose-milk gave a significantly

TABLE 1

Effect of kind of food, frequency of feeding and water on survival of flies
Accumulative mortality (number of flies dead)

Observation Period	Milk				Milk and Sucrose				Brain Heart Infusion			
	Water		Without water		Water		Without water		Water		Without water	
	Fq. 1	Fq. 2	Fq. 1	Fq. 2	Fq. 1	Fq. 2	Fq. 1	Fq. 2	Fq. 1	Fq. 2	Fq. 1	Fq. 2
½ day	0	0	0	0	0	0	0	0	0	0	0	0
1 day	0	0	1	0	1	0	0	0	0	0	0	0
1½ days	4	3	1	4	4	3	2	3	6	6	3	7
2 days	4	3	2	4	4	3	4	5	9	8	9	9
2½ days	8	6	7	8	7	8	10	9	10	10	10	10
3 days	8	6	7	8	7	8		9				
3½ days	8	7	8	9	9	9		9				
4 days	8	7	9	9	9	9		9				
4½ days	9	9	9	9	10	9		10				
5 days	9	9	9	9		9						
5½ days	9	10	10	10		9						
6 days	9					9						
6½ days	10					10						

Fq.1—Flies fed 2 times/day
Fq.2—Flies fed 3 times/day

longer survival time than Brain-Heart Infusion ($P < 0.05$). Flies given water lived longer, although not significantly longer, than those not given water. Three feedings a day did not yield a significantly longer ST_{50} than 2 feedings as shown in Table 1. The mean survival time on milk or sucrose milk was 2.2 to 2.5 days, on Brain-Heart Infusion, 1.7 to 1.8 days. The maximum survival time for any fly was 6.5 days. No interactions among foods, water and feeding frequencies were found to be significant. The data indicate that climatic conditions of 90°F. and 35 per cent relative humidity are not suitable for maintaining flies for prolonged experimental studies.

The test was repeated in an insectary maintained at 77°F. and 70 per cent relative humidity. Again milk and sucrose-milk gave a significantly higher survival time than did Brain-Heart Infusion (9.5 and 9.8 vs. 3.2 days). However, as before, there were no significant differences between the ST_{50}'s of milk and sucrose-milk. In this test the frequencies of feeding were once and twice a day instead of 2 and 3 times per day. In no case did feeding twice a day give a significantly greater ST_{50} than did 1 feeding per day. Survival time was significantly greater in each case than it was in the previous test. Again, there was no significant interaction among the various conditions.

Mortality of the housefly under laboratory conditions. In some preliminary observations it was found that the ingestion of *E. coli* did not significantly change the survival time of immobilized flies. The following experiment was set up to give some idea of the comparative survival of the insects under more natural conditions.

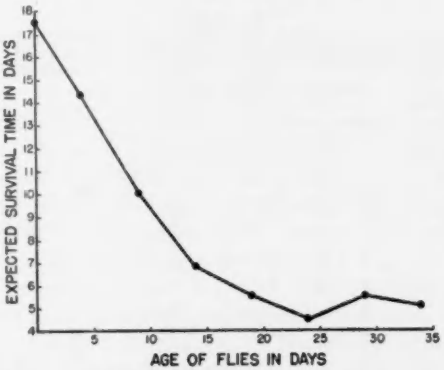


FIG. 1. Survival times for insectary maintained flies.

Two cages containing respectively 232 and 235 flies of mixed sex were set up and observed for mortality rates. All flies were within 2 hours of the same age. They were taken out of the rearing jars and put in standard 1 cu. ft. holding cages at 77°F., 70 per cent relative humidity, and given water, powdered milk and sugar.

A plot of the cumulative mortality, in per cent, with respect to the day of observation, gave a linear relation. Although a significant difference was found between the 2 replicates, it was thought that the combination would yield a better laboratory estimate of the mortality rates and expected survival times. The expected survival times (for 50 per cent of the flies remaining at any given age) are inversely related to the age of the fly, as shown in Figure 1.

The fate of E. coli *ingested by individual flies.* Known numbers of *E. coli* were fed to individual immobilized flies by means of a calibrated micrometer-driven syringe. Daily checks were made for the recovery of the organism as previously described. In this manner we were able to quantitate the rate of excretion of the bacteria. The following controls were run: (1) a number of flies received no *E. coli*. This was done to determine if any of the organisms were already present in the fly or if we were picking up any of the organisms from extraneous sources; and (2) a known number of *E. coli* was dropped into the saline blanks under mounted non-infected flies in order to judge the probable fate of the organisms deposited in the collection tubes by infected flies.

The results from 76 flies given 2.1×10^6, and 18 flies fed 1.0×10^6 bacteria per fly are shown in Tables 2 and 3. The greatest number of bacteria were recovered 48 to 72 hours after feeding. Somewhat over 50 per cent of the bacteria fed were recovered. Death of some flies may have lowered this figure somewhat. However, rarely did a fly die before 48 hours. The mortality is indicated in the last column of Tables 2 and 3. In some instances an individual fly would continue to discharge a large number of organisms for several days and, occasionally, the number excreted would be 2 or 3 times greater than the number fed, although never as much as 10x. The controls showed that fewer organisms were recovered from

TABLE 2

Bacterial output by individually fed flies (dosage 8.1 × 10^6)

Days	Number of organisms excreted by the flies						Total
	0–10^3	10^3–10^3	10^3–10^4	10^4–10^5	10^5–10^6	10^6 and above	
	Number of flies						
1	11	11	23	15	16	0	76
2	8	13	22	17	6	9	75
3	23	22	13	7	4	5	74
4	38	6	7	10	1	8	70*
5	36	16	6	9	2	2	71
6	37	8	8	8	1	3	65
7	27	14	4	4	4	3	56
8	30	4	5	3	2	3	47
9	31	7	2	1	0	2	43
10	35	1	1	0	0	1	38
11	28	0	2	0	0	0	30

* Counts not available for 2 specimens which were not included.

TABLE 3

Bacterial output by individually fed flies (dosage 1.0 × 10^6)

Days	Number of organisms excreted by the flies						Total
	0–10^3	10^3–10^3	10^3–10^4	10^4–10^5	10^5–10^6	10^6 and above	
	Number of flies						
1	5	3	6	4	0	0	18
2	6	2	5	2	1	1	18
3	3	7	2	2	0	3	17
4	5	2	3	3	0	2	15
5	8	2	3	1	0	1	15
6	8	0	1	2	0	0	11
7	4	1	4	0	0	0	9
8	4	1	1	0	0	0	6
9	3	0	0	1	0	1	5
10	2	0	0	0	0	0	2
11	0	1	0	0	0	0	1

the vials after 24 hours than were initially put into them, and that there was no contamination from the flies or from extraneous sources. We interpret the failure of the organisms to multiply in the saline blanks to mean that the fly droppings did not provide a sufficient concentration of nutrient material to maintain growth. It is conceivable that in a smaller volume of saline the bacteria would have undergone some multiplication (Wilson, 1952).

While we were primarily interested in the number of organisms deposited by the flies, the number of organisms remaining in the insect immediately after death was checked and ranged from 0 to 10^5, depending upon the length of time the fly lived. If it lived beyond 6 days, usually only a few or no streptomycin-resistant *E. coli* remained.

The effect of different concentrations of sucrose on the propagation of E. coli *and*

survival of the flies. In the previous experiment there was no definite evidence of multiplication of the ingested organisms. It was considered that the type of food might be important for retention and possible multiplication of bacteria in the insects. Although sugars have been reported as important for the survival of flies (Glaser 1923, 1924), it is conceivable that the fly might ingest a concentration of sucrose that would not favor the growth of certain microorganisms. Milk is considered a good nutritional base for both flies and bacteria. Therefore, skim milk plus various concentrations of sucrose were used to test the effect of diet. Five concentrations of sucrose were used (0.1, 0.2, 0.3, 0.4, and 0.5 M). Each solution (double strength) was mixed 1:1 with a 24-hour culture containing the desired number of *E. coli*. These suspensions were each fed to 20 mounted flies as previously described. Both flies and bacteria were then handled in the same manner as in the previous experiment.

The considerations here were: (1) total bacteria disseminated per fly for each treatment; (2) the time in days for 50 per cent mortality of the flies (ET_{50}); and (3) cumulative per cent of total bacterial count per day, which was used to indicate, in a qualitative manner, the relationship between bacterial output and longevity.

The results from the 2 tests are shown in Table 4. In Test 1 each fly was given 2.6×10^6 bacteria; flies in Test 2 were given 1.5×10^6 organisms each. Since the variation between the mean total count of *E. coli* for different diets did not exceed the average variation of the total count within diets, the hypothesis of equal mean log total bacterial count for all the diets was accepted. This does not mean that there were no measurable differences. However, if a true difference between the mean total counts for the 5 treatments exists, it was not detected with a sample of 20 flies per diet. In Test 1 the hypothesis that the flies for the 5 treatments came from a homogeneous population, with respect to mortality, is

TABLE 4

Mean total count (TC) of E. coli *recovered and 50 per cent mortality (ET_{50}) based on 20 flies per diet*

Diet	Mean TC $\times 10^4$	ET_{50} (days)	Variance (ET_{50}) $(s.d.)^2$
Test 1 (flies fed 2.6×10^6 bacteria)			
(A) 0.1 M suc. in milk	8.3	7.7	3.1
(B) 0.2 M suc. in milk	9.3	10.7	7.0
(C) 0.3 M suc. in milk	19.3	10.9	12.4
(D) 0.4 M suc. in milk	19.6	12.5	25.5
(E) 0.5 M suc. in milk	3.9	8.1	8.2
Test 2 (flies fed 1.5×10^6 bacteria)			
(A) 0.1 M suc. in milk	8.5	9.3	6.7
(B) 0.2 M suc. in milk	4.4	8.5	15.1
(C) 0.3 M suc. in milk	7.2	9.6	10.4
(D) 0.4 M suc. in milk	17.5	10.5	13.3
(E) 0.5 M suc. in milk	4.2	11.1	41.2

rejected due to the differences in variance of the 5 samples. This invalidates a statistical comparison of ET_{50}'s. In Test 2 there was no significant difference between the mean log total counts or ET_{50}'s for the 5 diets.

Plots of cumulative per cent of total bacterial count and of mortality per day against time in days were both more or less linear for all tests. Almost all the bacteria in each treatment of Test 1 were recovered by the ET_{50}. All of the bacteria in each case in Test 2 were excreted by the ET_{50}. In other words, under these test conditions the fly outlived its usefulness as a vector of the test organism.

The use of radioactive organisms to study the bacteria-fly relationship. There are many reports of tagging bacteria and viruses with radioactive substances (Labaw *et al.*, 1950; Harper and Morton, 1952; and others). Also, many insects have been rendered radioactive, without apparent harmful effects, by being fed radioactive material with their food (Hoffman *et al.*, 1951; Hassett and Jenkins, 1951; Fredeen *et al.*, 1953).

In this experiment, *E. coli* were treated with P^{32} as previously described and centrifuged for 30 min. at 4,000 rpm; they were then washed and centrifuged 3 times with 10 ml. of normal saline. Each time, the supernatant was checked for viable bacteria and radioactivity. It was found that we were not able to free the supernatant completely of bacteria with our centrifuging equipment. If we assume that our method of centrifuging and washing the bacteria freed the surface of the tagged organisms of non-bound radioactive material, then the bacteria were tagged with enough radioactive phosphorus to give approximately two disintegrations per minute (dpm) per bacterium.

In some preliminary tests the radioactive bacteria appeared to lose some of their P^{32} to the host. P^{31} (non-radioactive) was added to the food of the larval and/or adult flies in an attempt to prevent the loss. The P^{31} solution was prepared as follows: 3.0 grams of K_2HPO_4 were dissolved in 437 ml. of distilled water; 0.3 gm. of KH_2PO_4 was dissolved in 63 ml. of distilled water. The two solutions were mixed and 1 ml. was added to 1 ml. of 1 M sucrose-milk solution which was then fed to the adults; in the case of the larvae the phosphorus solution was added directly to the horsemeat.

The P^{31} solution was given as follows: (A) to neither larvae nor adults, as a control; (B) to both larvae and adults; (C) to larvae and not to adults. A comparison of the total radioactive counts per fly for the 3 treatments indicates that treatment B gave a significantly higher mean total count than did treatments A and C which were not significantly different from each other. A similar analysis for total bacterial counts indicates that there were no significant differences between the mean total bacterial counts for the 3 conditions. In order to determine the incidence of bacterial and radioactive output per day for each treatment, the sums of the log bacterial counts per fly per day and log radioactive counts per fly per day were plotted (Fig. 2). Generally speaking, these curves show that the bacterial count is somewhat errant and by the sixth day drops to a negligible output. The radioactive count shows a continuous decrease for about 6 or 7 days and persists longer than do viable bacteria.

The coefficient of correlation between the average number of bacteria and

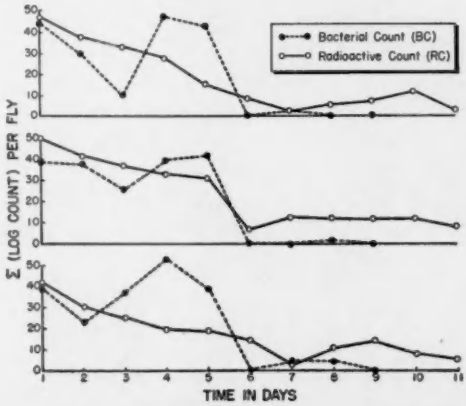


FIG. 2. Bacterial and radioactive output of flies fed P^{32}-tagged *E. coli*. Upper—Neither larvae nor adults were given P^{31}. Center—Both larvae and adults were given P^{31}. Lower—Larvae were given P^{31}, but the adults were not.

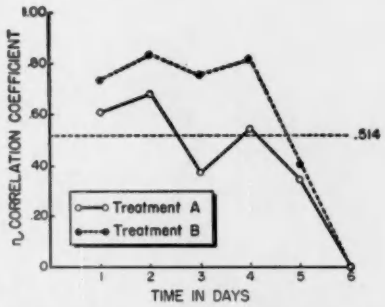


FIG. 3. Coefficient of correlation of bacterial and radioactive counts from flies fed P^{32}-tagged *E. coli*. (A) Neither larvae nor adults were given P^{31} in their food. (B) Both larvae and adults fed P^{31} with their food. Values above 0.514 are significantly different from 0, $p = 0.05$.

radioactive counts was significant beyond the 0.05 level of probability with treatments A and B for several days (Figure 3). However, there were individual cases where there was no correlation. In some instances fecal collections were without observable radioactive counts, and yet, if the fly died or was sacrificed, it showed a very high radioactive count. On the other hand, flies whose excreta showed no bacterial count never gave observable bacterial counts when they died or were sacrificed.

The addition of P^{31} to the diets apparently had no adverse effect on the longevity of the insects and did not influence the bacterial or radioactive counts in this manner. An analysis of the time to death for the 3 treatments indicates that the average time to death did not differ significantly. On the basis of these observations, the use of radioactively labeled bacteria shows promise of being useful

TABLE 5

The rate of uptake of bacteria by flies from infective baits under laboratory conditions

Time after baits were placed in cage with the flies	Average numbers of organisms/fly	
	Test 1	Test 2
15 Mins.	6.4×10^5	2.9×10^6
30 Mins.	1.9×10^6	2.9×10^6
45 Mins.	1.7×10^6	7.7×10^6
60 Mins.	3.2×10^6	5.3×10^6
90 Mins.	2.8×10^6	—
150 Mins.	1.7×10^6	—
270 Mins.	1.6×10^5	—
390 Mins.	6.6×10^5	3.2×10^6

in dissemination studies; but, because of the technical defects noted above, the results reported here cannot be regarded as final. More work along these lines is needed.

Rate of uptake of bacteria by the flies from an infective source under laboratory conditions. In this test approximately 230 flies of the same age and of mixed sex were isolated in a cage within 2 hours of emergence. They were starved for the next 24 hours, then given an infective bait seeded with a 24-hour culture of *E. coli*. A random sample of from 10 to 15 flies was homogenized in normal saline and serial dilutions were plated. The process was repeated at various intervals ranging from 15 minutes to 2 hours (Table 5). The results indicate that the insects obtained a maximum number of bacteria from the infected source in 45 to 60 minutes; and that continued exposure up to 6.5 hours did not result in an increase of bacteria. After approximately 2.5 hours there was an apparent drop in the number of bacteria but after approximately 4.5 hours there was again an apparent rise in the number of organisms. These results could be interpreted to mean that after the fly takes a full engorgement of organisms, some of the latter are lost by regurgitation or defecation. However, after about 4.5 hours the flies begin to feed again and by so doing increase the number of organisms contained within their bodies. Control flies fed on an uninoculated bait were negative for the test organism.

DISCUSSION

Among the numerous species of arthropods commonly reared for experimental work, there are few on which there is sufficient information about optimal conditions for propagation and survival. With the housefly, there has been disagreement among workers in the field of bionomics. Thus, Tischler (1931), Hawley *et al.* (1951), and others report that the flies should be fed 2 or more times a day, yet Glaser (1923) stated that nothing was gained by feeding the flies more than once daily. In a number of transmission studies, Melnick and Penner (1947, 1952) fed the flies but once a day. Our observations appear to support this practice.

It seems important that more attention should be given to the conditions of food, frequency of feeding and storage in arthropod transmission studies. Natural

foods, when these are known, obviously would be preferred. However, in many cases the problems of contamination would be almost insurmountable. Food ingested by insects undoubtedly changes their internal environment, either by its own composition or as the result of the secretion of digestive juices. The frequency of this event could conceivably influence the alimentary flora. Storage of the experimental vectors can create many problems. The optimum climatic conditions for the organism and host are not necessarily the same. This fact is, demonstrated by the experimental transmission of filariasis by *Culex quinquefaciatus*, (Basu and Rao, 1937; and in transmission of Eastern equine encephalomyelitis by *Aedes aegypti* (Ingram and Collins, 1955 unpublished data), where it was found that holding the insects at 35°C. instead of 26°C, significantly increased the virus titer, but was unfavorable for mosquito survival.

The literature on the length of time the fly may retain ingested bacteria is also in disagreement (see, for examples, Hewitt, 1914). Most of the previous work has not been quantitative. From our study it would seem that the common coliform organism, *E. coli*, does not multiply in the alimentary canal of the fly, for we found the greatest number of *E. coli* were discharged within 48 to 72 hours, the approximate time required by the fly to excrete an inert substance such as carmine dye (Block, 1952 personal communication). Failure of introduced organisms to become established in arthropods may be attributed to unfavorable physical conditions such as temperature and pH, antagonism between two or more organisms present in the same environment (Sarkar and Tribedi, 1953; Henle, 1950; Ingram, 1954, unpublished data; and others) and other causes. It is also possible that diets other than our mixture of sugar and milk, or combinations of a number of other factors, might have allowed multiplication of the test organism within the fly, for example, by altering the composition of the intestinal flora and fauna. Such possibilities, as yet untested, emphasize the need for further study of the many intrinsic factors that might affect the transmission of disease organisms by flies. The effects of the equally numerous extrinsic factors, such as climatic conditions, on the fly-organism relationship have also received little attention, but are obviously important.

Solution of some of these problems should be facilitated by the methods tested in the present study. The micrometer syringe allows one to give the fly a known volume of material with a high degree of accuracy, and avoids dilution of the test organism by regurgitation, which may easily occur with other methods of feeding. The use of radioactively labeled bacteria has also demonstrated promise, though further work will be required to define the limitations of this technique. The data reported here are indicative of the kind of information that can be obtained with such methods, and will be extended in an attempt to provide answers to the many questions that still remain in the problem of the dissemination of disease organisms by flies.

SUMMARY

A number of conditions which may influence the survival of the fly were studied under experimental laboratory conditions. One feeding a day of milk

and sucrose was found to be sufficient for maintaining the flies. Water, when offered, was rejected most of the time and did not significantly increase the survival time (ET_{50}). Caged flies under normal insectary conditions lived considerably longer than did immobilized test flies.

In general, multiplication of *E. coli* did not appear to take place in the insect's alimentary canal. Somewhat over 50 per cent of the bacteria ingested were recovered. There was no significant difference in bacterial output by the flies fed on diets containing different amounts of sucrose in skim milk, although this would have had to be great in order to be significant because of the variations in the total counts within the diets. Almost all the bacteria were released by the time of the ET_{50}.

E. coli was tagged with radioactive P^{32} and fed to the flies. For several days thereafter there was fairly good correlation between the radioactive and bacterial counts.

The flies' rate of uptake of *E. coli* was determined under laboratory conditions. The maximum number of bacteria was found after 45 to 60 minutes. Following this, there was an apparent decrease for approximately 5 hours; then there was an apparent increase, indicating a revisit by the fly to the source of the organisms.

ACKNOWLEDGMENT

The authors are profoundly grateful to Dr. Leigh E. Chadwick for his contributions in the preparation of the manuscript, and to Messrs. I. A. DeArmon and Todd M. Fraizer for the tremendous amount of statistical work they performed on the data. Mr. James Bowman furnished much valuable technical help, especially with the radio isotope work. We are also indebted to Drs. Dale Jenkins and A. S. West for their criticisms of the manuscript.

REFERENCES

Basu, B. C., and Rao, S. S., 1937. Studies on filariasis transmission, *Ind. J. Med. Res.* **27**: 233–249.

Chadwick, L. E., Lovell, J. B., and Egner, V. E., 1953. The effect of various suspension media on the activity of cholinesterase from flies, *Biol. Bull.* **104**: 323–333.

Glaser, R. W., 1923. The effect of food on longevity and reproduction in flies, *J. Exp. Zool.* **38**: 383–412.

Hawley, J. E., Penner, L. R., Wedberg, S. E., and Kulp, W. L., 1951. The role of the housefly *Musca domestica* in the multiplication of certain enteric bacteria, *Am. J. Trop. Med.* **31**: 572–582.

Henle, W., 1950. Interference phenomena between animal viruses: a review, *J. Immunol.* **64**: 203–236.

Hewitt, C. G., 1914. *The housefly, Musca domestica* Linn. *Its structure, habits, development, relation to disease and control*, Cambridge, England.

Melnick, J. L., and Penner, L. R., 1947. Experimental infection of flies with human poliomyelitis virus, *Proc. Soc. Exp. Biol. Med.* **65**: 342–346.

Melnick, J. L., and Penner, L. R., 1952. The survival of poliomyelitis and Coxsackie viruses following their ingestion by flies, *J. Exp. Med.* **96**: 255–271.

Sarkar, J. K., and Tribedi, B. P., 1953. Antagonism between *Vibrio cholerae* and *Bacterium coli*, *Ind. J. Med. Sci.* **78**: 403–408.

Tischler, N., 1931. A satisfactory nutrient for adult houseflies, *J. Econ. Ent.* **24**: 559.

West, L. S., 1950. *The Housefly*. Comstock, Ithaca, N. Y.

THE RELATIVE EGG PRODUCING CAPACITY OF *SCHISTOSOMA MANSONI* AND *SCHISTOSOMA JAPONICUM*[1, 2]

DONALD V. MOORE[3] AND JACK H. SANDGROUND[4]

Department of Preventive Medicine, New York University College of Medicine

Because an essential role in the pathogenesis of schistosomiasis has been ascribed to the parasite's eggs, the differential in the egg producing capacity of *Schistosoma mansoni* and *Schistosoma japonicum* has on several occasions been emphasized. Illustrating the comparative fecundity of two species, Brumpt (1930) states that the females of *S. mansoni* produce from 157 to 191 eggs per day (6 to 8 per hour) and that the females of *S. bovis* produce from 25 to 111 eggs per day (1 to 4 per hour). Unfortunately, Brumpt provided no data to substantiate this estimation nor did he show how he had arrived at these specific figures. Faust *et al.* (1934) made an effort to determine the egg output per female of *S. mansoni* in monkeys by "counting all of the eggs in the first and last five per cent of a suspension of entire daily fecal samples" and subsequently determining the number of female worms present in the host at autopsy. These authors state that they had made no attempt to determine the number of eggs trapped in the various tissues of the host but they noted that scrapings from areas 2 cm. square of the gut revealed "tens of thousands of eggs". From this they inferred that only a very small fraction of the eggs deposited by the female worms escaped by way of the feces. Although they made no actual attempt to determine, quantitatively, the egg producing capacity of *S. mansoni* these investigators concluded that each female produces from one to several hundred eggs per day.

In view of the lack of experimental evidence to support the contention that *S. mansoni* produces fewer eggs than *S. japonicum*, it was deemed important to verify this relationship and if possible to establish it quantitatively upon a firmer basis than has heretofore prevailed. To this end we have devised a procedure for counting, with what we believe to be a fair degree of accuracy, the egg output of the female schistosomes from the time of their maturation to the time the infection is brought to termination.

MATERIALS AND METHODS

Hamsters were selected as the mammalian host in this study because it has been experimentally shown by many investigators (Cram and Figgat, 1947; Moore *et al.*, 1949, 1954; and others) that the hamster is a favorable host to the three species of human schistosomes.

[1] This investigation was supported by research grant G-385 from the National Institutes of Health, Public Health Service.

[2] The authors gratefully acknowledge the technical assistance of Mr. Richard Crovatto and Miss Christa Kallman.

[3] Present address: Department of Microbiology, The University of Texas Southwestern Medical School, Dallas, Texas.

[4] Present address: 390 First Avenue, New York, N. Y.

Prior to exposure to infection the hair was closely clipped from the entire body of the hamsters except the head, after which the animals were placed in a battery jar containing about 2 inches of water for one hour in order to promote elimination of the contents of the bladder and bowels.

For each infection of *S. mansoni*, cercariae were obtained from a pool of at least 20 infected *Australorbis glabratus* (Puerto Rican strain). On standing, the cercariae tend to concentrate near the surface of the water from which they are easily removed with a capillary pipette. Small drops of water containing cercariae were placed on a 22 x 40 mm. plastic coverslip, counted under a binocular dissecting microscope and then carefully washed with a jet of spring water into a battery jar containing about an inch of spring water. The animals were kept standing in the exposure jar for one hour. Hamsters were exposed to either 75 or 150 *S. mansoni* cercariae each.

Cercariae for the exposure of hamsters to *S. japonicum* were obtained from 5 to 17 experimentally infected *Oncomelania formosana*. Cercariae of *S. japonicum* were removed by means of a hair loop from the surface film of water in which they were shed and transferred to a coverslip for counting with the aid of a binocular dissecting microscope. After counting, the coverslips were carefully placed in the bottom of the exposure jars and spring water added to a depth of about an inch. Hamsters were exposed to 75–80 cercariae each and allowed to remain in the exposure chamber for one hour.

Following exposure to cercariae the animals were caged individually and maintained on a regular laboratory "chow" diet until approximately 5 days before the anticipated first appearance of eggs in the feces. At this time individual animals were transferred to a wire cage with a ¼″ wire mesh bottom and this was placed in a pan containing physiological salt solution to the depth of ½ inch. From preliminary studies we had found that the regular laboratory "chow" contains large quantities of indigestible plant fibers which naturally interfere with the accuracy of egg counts. After considerable experimentation it was found that this handicap could be overcome by supplanting "chow" with a diet of cottage cheese fortified with Brewer's yeast. The fecal output on this diet was largely free from solid particles of a size or shape which would mask the eggs.

Care was taken to see that all fecal pellets passed through the wire mesh cage bottom into the saline solution. Feces were collected for processing on a rigid 24 hour basis. To remove the hairs which were always present, the saline from the cage pan was poured through a fine sieve. The fecal pellets were then washed from the cage pan into a coarse wire sieve and pressed through the sieve with a round-end glass rod into the saline from the pan. The entire fecal suspension was then poured through a fine mesh wire sieve into a two liter beaker. Any fecal particles which would not freely pass through the fine wire sieve were broken up further with a glass rod; finally the sieve received a thorough rinsing with fresh saline.

The fecal suspension was allowed to stand for 1 hour, after which the supernate was siphoned off to a level approximately 2 inches above the sediment and the beaker refilled with saline. This process was repeated 3 to 4 times to clarify the suspension, then the sediment and remaining supernate were transferred by

means of a pipette to a 50 ml. graduated conical centrifuge tube. The sediment was then concentrated by centrifugation and the supernate discarded. The packed sediment was diluted with a 0.01 % eosin saline solution to the 3.75 ml. or 7.5 ml. mark depending on the dilution desired. It was found that the dilute eosin solution greatly facilitated the counting of eggs (especially viable eggs) under the microscope. The eosin stained all fecal debris a light pink and afforded a useful contrast for the detection of eggs.

Prior to and during the withdrawal of a sample for counting, a slow but steady stream of air was bubbled through the suspension to insure an even distribution of eggs. Four samples of 0.075 ml. were withdrawn by means of a Stoll pipette, placed on a 2 x 3 inch microscope slide and covered with a 24 x 50 mm. coverglass. The preparations were counted with a compound microscope under 60 × magnification. The authors and two experienced technicians participated in all egg counts. If there was significant variation between individual results additional samples were counted. These counts were averaged and multiplied by the dilution factor to determine the number of eggs passed in the 24 hours. At the beginning of egg passage a dilution factor of 50 was used and as the number of eggs in the feces increased higher dilutions were required. In every instance fecal specimens were collected and examined for eggs from 2 to 5 days before the first eggs appeared in the feces. Using the above described procedure it was possible to detect the passage of as few as 12 eggs in a 24-hour fecal sample.

The aim of this study was to determine as accurately as possible the total number of eggs produced by the female worms. Since eggs deposited in certain tissues of the host are ultimately destroyed, it was arbitrarily decided to terminate the infections 21 days after the onset of egg passage. In some cases it was necessary to terminate the infection earlier because the animal had developed a severe diarrhea or had become moribund as a consequence of a heavy worm burden.

The hamsters were killed with ether and the worms recovered by the perfusion technic of Yolles *et al.* (1947). Worms were counted and classified in three categories; mature males, mature females and immature worms. If there was any doubt as to the maturity of the worms, they were fixed in alcohol-formol-acetic acid, stained with Mayer's carmalum and mounted on slides for critical microscopic examination.

After the completion of the perfusion, a double ligature was placed between the pyloric end of the stomach and the duodenum and another on the ileum at the ileocecal junction. The small intestine was then removed by cutting between the ligatures and carefully severing the intestine from the mesentery close to the intestinal wall. The ligatures were next removed, a cannula inserted into one free end of the intestine and its contents flushed into a beaker by a stream of citrated saline solution. The cecum and large intestine were in like manner freed of their abdominal attachments and a cannula inserted into the rectum and a small incision made in the tip of the cecum; the contents of the large intestine and cecum were flushed with a stream of citrated saline into a beaker containing the contents of the small intestine.

The total number of eggs present in the intestinal contents was determined in

the same way as in the feces, and the eggs found were added to the total eggs passed in the feces. After flushing, the large intestine and cecum were removed by cutting as close to the anus as possible. The spleen was separated from the mesentery and removed. Next, the remaining mesenteries, mesenteric lymph nodes and pancreas were removed from the abdomen by cutting close to the dorsal attachments.

By a digestion procedure the eggs contained in the small intestine, large intestine including cecum, liver, lungs, spleen and the mesenteries with their associated lymph nodes and pancreas were counted. The organs were minced with a razor blade on a block of heavy simulated leather plastic material. The finely divided tissue was washed into separate beakers of appropriate size, (250 ml. or 100 ml. depending upon the amount of tissue) with a digestive solution (1% pepsin, 0.5% HCl made up in 0.85% NaCl solution). The beakers were placed in a 37°C. water-bath and the contents stirred continuously for 4 or 5 hours. They were usually allowed to remain overnight in the water-bath without stirring, then agitated for a short period the following morning or until digestion of the tissue appeared to be complete.

After digestion the contents of the beakers were transferred to 50 ml. or 15 ml. graduated conical tubes, centrifugated, and the sediment thoroughly mixed with a 0.1% solution of NaOH to remove any remaining tissue. The suspensions of eggs freed from the tissues were diluted and counted in the same manner as the fecal specimens with the exception that 0.1% NaOH was used as the diluent. It was found that higher concentrations of NaOH produced a distortion and breakage of large numbers of egg shells. In every instance where dilution counts were made, four samples of 0.075 ml. were counted. The number of eggs found in the lungs and spleen were so few that a dilution count was not made but the sediments from these organs were concentrated by centrifugation and a total egg count made.

It is difficult to ascertain with accuracy the day on which the female worms produce their first eggs. It was felt that it would be adequate to regard the day on which eggs first appeared in the feces as the basis for the calculation of daily egg output per female worm.

Data on the maturity and sex of the worms recovered from individual hamsters used in this study are presented in Table 1. The percentage recovery (worms/cercariae) for the 6 animals infected with *S. mansoni* averaged 61 per cent, and 46 per cent for the 5 animals infected with *S. japonicum*. The greater variation in the percentage recovery in *S. japonicum* infections is probably due to the difficulties involved in the manipulation of the cercariae of this species. The number of female worms exceeded the number of males in every animal except numbers 5 and 7 infected with *S. japonicum* where equal numbers of males and females were found. As is to be expected in animals with an excess of female worms, the unpaired worms were sexually immature.

The distribution of the eggs in the feces and the various organs and tissues examined is shown in Table 2. These figures were rounded to the nearest 10 and the averages shown for the *S. mansoni* and *S. japonicum* animals to the nearest

TABLE 1

Number and sex of worms recovered from hamsters after exposure to S. mansoni *and* S. japonicum cercariae

Animal Number	Number Cercariae	Worms Recovered		Sex of Worms		
		Number	Percentage	Mature ♂♂	Mature ♀♀	Immature
Schistosoma mansoni						
I	75	36	48.0	18	18	—
II	150	58	38.6	29	29	—
X	150	98	65.3	45	45	8
XII	150	112	74.6	52	52	8
XVII	75	66	88.0	17	17	32
XVIII	75	42	56.0	13	13	16
Total.....	675	412				
Average percentage worm recovery 61.0						
Schistosoma japonicum						
1	80	60	75.0	13	13	34
2	80	44	55.0	8	8	28
5	75	34	45.3	16	18	—
7	75	10	13.3	3	7	—
8	75	31	41.3	7	7	17
Total.....	385	179				
Average percentage worm recovery 46.4						

100 with the exception of the average eggs found in spleen and lung which were rounded to the nearest 10.

The prepatent period for *S. mansoni* and *S. japonicum* (Formosan strain) in the hamster is essentially the same as indicated in Table 2. On the average, eggs of *S. mansoni* first appeared in the feces on the 46th day and eggs of *S. japonicum* on the 42nd day. It is of interest to note that in the *S. mansoni* animals, eggs containing fully developed miracidia were usually seen on the first day of egg passage, while in the *S. japonicum* infections eggs containing miracidia were usually not seen until the fourth or fifth day after egg passage had commenced. The first eggs passed in the feces in *S. japonicum* infections were usually abnormally small and irregular in shape, and the shells were either empty or contained an amorphous mass. The number of eggs appearing in the feces increased rapidly for 7 to 10 days and then reached a plateau which fluctuated only slightly for the remainder of the period of examination. Fluctuations in daily egg counts were greater in the animals infected with *S. japonicum*. This can be explained by the well established fact that many *S. japonicum* eggs are usually expelled from the uterus of the female at one time. This produces clusters of eggs which are

TABLE 2

Distribution of eggs found in hamsters infected with S. mansoni *and* S. japonicum

Animal No.	First eggs found in feces (days)	No. mature females	Total eggs produced	Total eggs and percentage according to location						
				Feces	Large intestine	Small intestine	Liver	Mesentery	Spleen	Lung
Schistosoma mansoni										
I	47	18	155,910	31,950 (20.5%)	27,600 (17.7%)	60,800 (39.0%)	35,400 (22.7%)	—	30 (0.02%)	130 (0.08%)
II	44	29	303,900	86,680 (28.5%)	70,800 (23.3%)	77,200 (25.4%)	69,200 (22.7%)	—	10 (0.004%)	10 (0.003%)
X	42	45	212,220	31,650 (14.9%)	38,800 (18.3%)	75,600 (35.6%)	61,600 (29.0%)	4,300 (2.0%)	200 (0.09%)	70 (0.03%)
XII	43	52	244,030	32,460 (13.3%)	56,800 (23.3%)	81,600 (33.4%)	68,800 (28.2%)	3,800 (1.5%)	290 (0.1%)	280 (0.1%)
XVII	44	17	94,020	27,760 (29.5%)	7,600 (8.1%)	24,400 (25.9%)	25,200 (26.8%)	8,700 (9.3%)	350 (0.3%)	10 (0.01%)
XVIII	44	13	102,030	29,900 (29.3%)	5,300 (5.2%)	38,000 (37.2%)	24,800 (24.3%)	3,810 (3.7%)	200 (0.2%)	20 (0.02%)
Average	46	29	185,400	40,100 (22%)	34,500 (18%)	59,600 (32%)	47,500 (26%)	3,400 (2%)	180 (0.1%)	100 (0.05%)
Schistosoma japonicum										
1	45	13	860,490	143,840 (16.7%)	413,650 (48.0%)	116,700 (13.6%)	168,600 (19.6%)	16,950 (2.0%)	60 (0.007%)	690 (0.08%)
2	44	8	452,240	43,490 (9.6%)	231,200 (51.1%)	41,800 (9.2%)	128,200 (28.3%)	6,840 (1.5%)	550 (0.1%)	160 (0.04%)
5	42	16	907,410	214,990 (23.7%)	425,800 (46.9%)	70,400 (7.8%)	181,600 (20.0%)	14,500 (1.6%)	10 (0.001%)	110 (0.01%)
7	39	3	217,450	19,820 (9.1%)	105,400 (48.5%)	20,000 (9.2%)	71,600 (32.9%)	580 (0.3%)	0	50 (0.02%)
8	40	7	400,200	29,510 (7.4%)	228,800 (57.2%)	45,200 (11.3%)	94,800 (23.7%)	1,010 (0.3%)	0	880 (0.2%)
Average	42	9	567,600	90,300 (16%)	281,000 (50%)	58,800 (10%)	129,000 (23%)	8,000 (1%)	120 (0.02%)	380 (0.06%)

eliminated from the intestinal wall into the lumen by the breaking through of egg abscesses.

Although the total number of eggs found in the feces and in other locations is given in Table 2, the percentages according to location of the total eggs produced, provide a better basis for comparison. The average percentages shown were calculated from the average total eggs in each column rather than from an average of the percentage figures given for individual animals. In *S. mansoni* infections an average of 22 per cent of the total eggs produced were passed in the feces while only 16 per cent of the *S. japonicum* eggs produced were passed in the feces. The data indicated that in *S. mansoni* hamster infections a greater percentage of the total eggs produced escape from the intestinal wall and are passed in the feces.

The greatest difference in the distribution of eggs in the tissues and organs of hamsters infected with *S. mansoni* and *S. japonicum* was seen in those recovered from the large intestine (including the cecum) as compared with the small intestine. In *S. mansoni* animals an average of 18 per cent of total eggs were found in the large intestine, while in the *S. japonicum* animals an average of 50 per cent of the eggs were found in this location. In animal number 5 of the *S. japonicum* series the cecum and large intestine were digested and the eggs counted separately. The cecum contained 177,500 eggs or 19.5 per cent of the total while the large intestine contained 248,300 or 27.4 per cent of the total. An average of 32 per cent of the total eggs was recovered from the small intestine of the *S. mansoni* animals while the same area in the *S. japonicum* animals yielded an average of 10 per cent.

Bearing in mind that all eggs found in the feces have been deposited in the tissues of the host's alimentary canal by the female worms, it is interesting to note that if the average percentages for feces, large intestine, and small intestine are added we find that 77 per cent of all *S. mansoni* eggs and 76 per cent of all *S. japonicum* eggs produced were deposited in the tissues of the alimentary canal.

The distribution of eggs between the small and large intestine gives some indication of the location of the mature worms. Faust *et al.* (1934) state that *S. mansoni* in man and in experimental animals shows a primary predilection for the posterior branches of the superior mesentric and the inferior mesenteric veins, and that it is for this reason that most of the intestinal lesions are to be found in the area drained by these veins. They point out that this is in contrast to *S. japonicum* which at the beginning prefers the anterior branches of the superior mesenteric and subsequently migrates posteriad. Our findings are not in accord with this conclusion, for they show that *S. mansoni* tends to concentrate in the veins draining the small intestine and *S. japonicum* in the veins draining the large intestine. It is quite possible, however, that the localization of the worms in certain areas of the alimentary canal may vary with the species of host. A more valid comparison cannot be made unless other species of host are examined and studied using a procedure comparable with the one used here.

Eggs located in the liver represent those which may have been carried to the

organ by way of vessels of the mesenteric system in addition to eggs which are laid by females residing in the intrahepatic vessels. In comparing the distribution of eggs of *S. mansoni* and *S. japonicum* in the host it may be noted that the difference in numbers of eggs found in the liver is not as great as the difference in eggs found in the large and small intestine. Thus in *S. mansoni* animals, an average of 26 per cent of the total eggs produced were recovered from the liver while in *S. japonicum* infections the corresponding figure is 23 per cent.

In Table 2 the column heading "Mesentery" refers to counts made from the pancreas, the mesenteries and mesenteric lymph nodes including retroperitoneal lymph nodes. In some animals the pancreas was observed to be hemorrhagic and tissue press preparations revealed the presence of eggs. Eggs were also seen in the mesenteric lymph nodes and the retroperitoneal lymph nodes. In *S. mansoni* animals the percentage of total eggs found in the "mesentery" ranged from 1.5 to 9.3 per cent (average 2 per cent)[5]. In *S. japonicum* animals the percentage in the mesenteries etc. ranged from 0.3 per cent to 2 per cent (average 1 per cent) of the total eggs produced.

The number of eggs found in the spleen and lung in both *S. mansoni* and *S. japonicum* infections was variable. In *S. mansoni* animals an average of 0.1 per cent of the total was found in the spleen and an average of 0.05 per cent in the lung. In the *S. japonicum* animals the average for the spleen was 0.02 per cent with 2 animals showing no eggs in the spleen. The average for the lung in the *S. japonicum* animals was 0.06 per cent.

Using the data presented in Table 3 showing the number of eggs per day per female for *S. mansoni* and *S. japonicum* and carrying the calculations further with regard to time, we find that on the average each female *S. mansoni* produced approximately 12 eggs per hour or one egg every 5 minutes and each female *S. japonicum* produced approximately 146 eggs per hour or more than 2 eggs per minute. This further emphasizes the marked difference in the egg producing capacities of the two species.

The question arises as to how soon after the appearance of eggs in the feces the female worm reaches the egg production levels found in the data presented on the above six *S. mansoni* and five *S. japonicum* hamsters. As stated earlier, some of our animals developed diarrhea and became moribund as a result of the heavy infection. These animals were not included in the above data because the distribution of eggs in the tissues and feces varied to a certain extent with the age of the infection. In one animal harboring 36 mature females and autopsied on the 5th day of egg passage it was found that each female produced an average of 450 eggs per day. This figure is somewhat higher than any of the figures given for individual animals in Table 3. One animal harboring 45 females was autopsied on the 8th day of egg passage, each female had produced an average of 309 eggs per day; another animal harbored 36 females and was autopsied on the 15th day of egg passage, each female had produced an average of 275 eggs per day. These last two figures are well within the range given for individual *S. mansoni* animals in Table 3. From these data we must conclude that *S. mansoni* females reach a peak of egg production as early as the 5th day of egg passage in the feces.

[5] When the average is calculated on the basis of the 4 animals in which these areas were examined the average is 3 per cent.

TABLE 3

Egg production of females of S. mansoni *and* S. japonicum *in hamsters*

Animal No.	No. Mature Females	No. Days Eggs Passed in Feces	Total Eggs Produced	Average Eggs per Female	Average Eggs Per Day per Female
			Schistosoma mansoni		
I	18	21	155,910	8,660	412
II	29	26	303,900	10,480	403
X	45	16	212,220	4,720	295
XII	52	16	244,030	4,690	293
XVII	17	29	94,020	5,530	190
XVIII	13	26	102,030	7,850	302
Average.....	29	22	185,400	6,400	291 (300)
			Schistosoma japonicum		
1	13	21	860,490	66,190	3,151
2	8	16	452,240	56,530	3,533
5	16	19	907,410	56,710	2,984
7	3	16	217,450	72,480	4,530
8	7	16	400,200	57,170	3,573
Average.....	9	18	567,600	63,000	3,500

In order to obtain some idea as to how long the female worms are able to maintain this high rate of egg production and to determine, if possible, whether an increase in the duration of the infection would bring about the destruction of eggs in the tissues, one *S. mansoni* hamster was autopsied after eggs had been passed in the feces for 83 days. This animal harbored 11 mature females which had produced a total of 242,170 eggs, an average of 22,020 eggs per female in 83 days or some 265 eggs per day. This is well within the range for eggs per day per female obtained in individual animals shown in Table 3 where eggs had been passed in the feces for an average of 22 days. The distribution of eggs in the feces and tissues of this animal was comparable with the findings reported for the *S. mansoni* animals in Table 2. In order to determine more accurately the effect of the duration of the infection upon the egg producing capacity of the females additional animals would have to be studied. The data obtained on this one animal indicate that the egg production of the female *S. mansoni* may be maintained at a fairly constant level for at least 83 days in the hamster and that in this period few if any eggs are destroyed beyond detection in the tissues of the host.

Considerable interest has been shown in the differences between the various strains of *S. japonicum*, more especially since the report of the existence in Formosa of a strain that does not affect man. In connection with the present study, in which the Formosan strain was used, we had the opportunity to examine one hamster infected with the Japanese strain. This hamster was exposed to 75 cercariae from naturally infected *O. nosophora* obtained through the courtesy of Dr. L. S. Ritchie of the 406th Medical General Laboratory in Tokyo. The

hamster was autopsied 57 days after exposure, by which time eggs had been passing in the feces for 21 days. Five mature female worms which the animal harbored had produced a total of 244,370 eggs, distributed as follows: feces, 25 per cent; large intestine, 44 per cent; small intestine, 7 per cent; liver, 22 per cent; mesenteries and associated lymph nodes and pancreas, 2 per cent; spleen, 0.002 per cent; lung, 0.01 per cent. Each female worm produced an average of 48,870 eggs or approximately 2,327 eggs per day. A comparison of these figures with those given in Tables 2 and 3 representing results obtained with the Formosan strain reveals that there is essentially no difference between the two strains with respect to distribution of eggs in the tissues and the egg producing capacity of the females.

SUMMARY

A procedure is described for the determination of the egg producing capacity of female schistosomes from the time of maturation to the termination of infection. Using this procedure the comparative egg producing capacities of female *S. mansoni* and *S. japonicum* were studied in the hamster and information obtained on the relative distribution of eggs in the feces and in the various tissues and organs.

Each female of *S. mansoni* produced an average of 300 eggs per day within the period under study. Only 22 per cent of all eggs produced were passed in the feces; 18 per cent remained in the wall of the large intestine; 32 per cent in the small intestine; 26 per cent in the liver; and 2 per cent in the mesenteries and its associated lymph nodes and pancreas. The percentage of eggs found in the spleen and lungs was small and numerically insignificant.

Each female *S. japonicum* produced an average of 3500 eggs per day. Only 16 per cent of all eggs produced were passed in the feces; 50 per cent were found in the wall of the large intestine; 10 per cent in the small intestine; 23 per cent in the liver and 17 per cent in the mesenteries, associated lymph glands and pancreas. As in the *S. mansoni* infections, the percentage of eggs found in the spleen and lungs was small and insignificant.

REFERENCES

BRUMPT, E., 1930. La ponte des schistosomes, *Ann. Parasit.* **8:** 263–297.

CRAM, E. B., AND FIGGAT, W. B., 1947. Experimental mammalian infection with the schistosomes of man. II. Comparative study of *Schistosoma mansoni* and *Schistosoma japonicum* infections produced by immersion and intraperitoneal injection, *N.I.H. Bull.* No. 189: 106–108.

FAUST, E. C., JONES, C. A., AND HOFFMAN, W. A., 1934. Studies on schistosomiasis mansoni in Puerto Rico. III. Biological Studies. The mammalian phase of the life cycle, *Puerto Rico J. Pub. Health and Trop. Med.* **10:** 133–196.

MOORE, D. V., AND MELENEY, H. E., 1954. Comparative susceptibility of common laboratory animals to experimental infection with *Schistosoma haematobium*, *J. Parasit.* **40:** 392–397.

MOORE, D. V., YOLLES, T. K., AND MELENEY, H. E., 1949. A comparison of common laboratory animals as experimental hosts for *Schistosoma mansoni*, *J. Parasit.* **35:** 156–170.

YOLLES, T. K., MOORE, D. V., DE GIUSTI, D., RIPSAM, C. L., AND MELENEY, H. E., 1947. A technique for the perfusion of laboratory animals for the recovery of schistosomes, *J. Parasitol.* **33:** 419–426.

INTRADERMAL REACTIONS TO *SCHISTOSOMA JAPONICUM* AND *S. MANSONI* ANTIGENS IN SCHISTOSOME DERMATITIS CASES[1]

H. F. HSÜ AND D. J. AMEEL

Department of Hygiene and Preventive Medicine, State University of Iowa, Iowa City, Iowa, and Department of Zoology, Kansas State College, Manhattan, Kansas

It has been well established that extracts of a variety of schistosomes, *S. mansoni*, *S. japonicum*, *S. spindale* and *S. bovis*, are useful for skin testing in human schistosomiasis (Fairley and Williams, 1927; Taliaferro and Taliaferro, 1931; Khalil and Hassan, 1932; Kan, 1936; Katzin and Most, 1946). It has also been demonstrated that even an extract of the cattle liver fluke, *Fasciola gigantica*, or of the frog lung fluke, *Pneumoneces medioplexus*, can be employed in the same way (Hassan and Betash, 1934; Culbertson and Rose, 1942). The question, however, as to whether schistosome dermatitis cases which are caused by non-human schistosome cercariae will react intradermally with schistosome extracts still remains unsettled. Cort (1936) was the first to suggest the skin test in schistosome dermatitis. At his suggestion, Hoffman used *S. mansoni* antigen on twenty unexposed subjects and all gave negative results. Cort, who had been exposed frequently to avian schistosome cercariae in the region of Douglas Lake, Michigan since 1914, gave a positive reaction. Macfarlane (1949), working on schistosome dermatitis in the New Zealand lakes, reported positive delayed-type skin reactions to the antigen made from a non-human schistosome cercariae, *C. longicauda*, in individuals who had been previously sensitized by exposure to this species of cercaria. He mentioned also that the immediate-type reactions shown in his cases were too slight to be of diagnostic value.

Augustine and Weller (1949) exposed rabbits to three species of avian schistosome cercariae, *C. stagnicolae*, *C. physellae* and *C. elvae*, and obtained no definite positive skin reactions with antigens made either from the cercariae of these avian schistosomes or from the cercariae of *S. mansoni*. In the same year, American workers in the 406th Medical General Laboratory in Japan (*Annual Report*, 1949) obtained negative intradermal reactions with antigens prepared from adult worms of *S. japonicum* in 51 persons, all of whom had had a history of *koganbyo*, a dermatitis caused by the cercariae of an avian schistosome, *Gigantobilharzia sturniae*. Positive intradermal reactions were obtained by Hsü *et al.* (1955, 1956) in their studies of a non-human strain of *S. japonicum* in Formosa, when an antigen prepared from a human strain of *S. japonicum* was used in an area endemic for a non-human strain of this parasite, and in human volunteers exposed to the cercariae of the latter. This paper presents additional data on this unsettled question.

[1] The present studies were made at the University of Michigan Biological Station, Cheboygan, Michigan, and were supported by a research grant, E.-939, P.T.M.(1), National Microbiological Institute, the National Institutes of Health, U. S. Public Health Service.

MATERIAL AND PROCEDURES

Forty-seven recent or old cases of schistosome dermatitis from Douglas Lake, Burt Lake, Mullet Lake, Black Lake, and Houghton Lake, all in Michigan, and two from West Okoboji Lake in Iowa, were studied. These cases included both males and females. Their ages varied from 8 to 67 years and their clinical manifestations varied from light to severe. Some of them had had eruptions during 1955, some in previous years, and some in both.

The *S. japonicum* antigen was a saline extract of the adult worms of the Japanese strain of this parasite. About 0.01 of the merthiolated antigen (1:10,000 dilution) and a merthiolated saline control were injected intradermally into the volar surface of the forearm. Reading of the test for an immediate-type reaction was done 15 minutes after injection, and an increase of 3 mm. in diameter of the wheal as compared with the control was considered a positive reaction. A second reading of the test for the delayed-type reaction was made 24 hours later, and the area of erythema, if any, was recorded.

The *S. mansoni* antigen was a merthiolated saline extract of the infected livers of the snail, *Australorbis glabratus*. The solution for the intradermal injection had a concentration of 1:100 w/v dried liver to saline. An extract of the same concentration made from the uninfected livers of these snails was used as a control in the test. The injections and readings were made in the same way as those for the *S. japonicum* antigens.

Forty-nine persons who had had no previous histories of schistosome dermatitis or had never been in the endemic areas of this disease were intradermally tested either with *S. japonicum* antigen alone or with both *S. japonicum* and *S. mansoni* antigens. Eight individuals were staff members of the University of Michigan Biological Station and 41 were medical students and medical staff members of the State University of Iowa.

RESULTS

Among the 49 schistosome dermatitis cases tested with *S. japonicum* antigen, three gave positive immediate-type reactions. One was W. W. Cort[2] who, as noted earlier in this paper, had been exposed a number of times while collecting snails in the cercaria-infested water of the Douglas Lake area and had had several severe attacks of dermatitis from 1914 to 1935. One was a man of 24, who had had a mild eruption one month before, and the other was a girl of 13 who had a similar history. In these three cases, the diameters of the wheals were 3.5 mm. greater than those of the controls. Especially in Dr. Cort's case, the wheal was accompanied by "pseudopodia" and a surrounding zone of erythema. Delayed-type reactions occurred in 36 cases. At the site of injection of the antigen, the area of the erythema in the delayed-type reactions varied from 0 to 13 mm. in diameter whereas it was only 0 to 4 mm. at the site of the injection of

[2] Dr. W. W. Cort was in China from 1923 to 1924 and Egypt during 1934. He informed the authors that he had no contact with sources of schistosome infection in China; in Egypt, where he was working on schistosomiasis, he had no history of infection.

the merthiolated saline. Dr. Cort showed a reading of 12 mm. at the site of injection of the antigen and 2 mm. at the site of the injection of the control fluid, and the other man showed 5 mm. vs 4 mm. No reading was made for the above-mentioned positive girl.

Forty-seven of the cases were also tested with the *S. mansoni* antigen. Only Dr. Cort and the girl previously mentioned showed positive reactions. The diameters of the wheals were 3 to 3.5 mm. larger than those of the controls. In a number of other cases there was little difference in the diameters of the wheals at the sites of injections of the extracts of the infected and the non-infected livers. Thirty-four of the cases were examined for delayed-type reaction. In these, the area of erythema following the injection of non-infected liver extract varied from 0 to 32 mm. and often reached the same extent or in a few cases became even greater than that resulting from the extract of infected livers, which varied from 0 to 23 mm.

All 49 non-exposed persons were negative for the immediate type of reaction to the *S. japonicum* antigen. The area of erythema shown in the delayed-type reactions varied from 0 to 6 mm. In one case, however, the extent of the erythema was 14 mm. in diameter, surpassing any obtained in the exposed cases.[3] The *S. mansoni* antigen was tested in only nine non-exposed persons. There were no positive immediate-type reactions. The size of the erythematous areas in persons giving delayed-type reactions varied from 0 to 9 mm. in diameter and were similar at the injection sites of the infected and non-infected snail liver extracts.

DISCUSSION

The results of our studies showed that of the 49 cases of schistosome dermatitis tested, only three (6 per cent) gave positive immediate-type reactions. Furthermore, it is possible that the positive reactions in these cases may be due to the hypersensitivity of the individuals to the foreign protein in the antigens. Although no positive reactors were found in the 49 non-exposed persons who served as controls in the present study, such a possibility still cannot be ignored. Delayed-type reactions were observed in a few cases, but were not significant. It is evident, therefore, that intradermal tests with the antigens from human schistosomes have no practical value in the diagnosis of schistosome dermatitis. The fact that schistosome dermatitis cases rarely give positive intradermal reactions to the antigens made from human schistosomes agree with those obtained by the 406th Medical General Laboratory workers (*Annual Report*, 1949) on the intradermal reactions of dermatitis cases caused by avian schistosome cercariae in Japan.

In certain circumstances, swimmer's itch is difficult to differentiate from other kinds of dermatitis. The etiology of the so-called seabather's eruption still remains unknown (Strauss, 1956), although schistosome cercariae shed from cer-

[3] This was a medical student, a native of Los Angeles, who a few years ago had swum in lakes in Minnesota, but claimed that he had never had any history of schistosome dermatitis or any itch after coming out of the water.

tain marine snails have been alleged to be a probable cause (Penner, 1950; Chu, 1952; Hutton, 1952; Stunkard and Hinchliffe, 1952). As dermatitis cases caused by avian schistosome cercariae do not usually give positive intradermal reactions to the antigens made from the human schistosomes, a differential diagnosis between schistosome dermatitis and non-schistosome dermatitis can not be made adequately by an intradermal test with human schistosome antigens. Such an attempt has been made in an effort to study the etiology of the seabather's eruption which occurred in April and May, 1954 in the area of Guatanamo Bay on the southeastern shore of Cuba (Strauss, 1956).

It is interesting to note that there was only an insignificant percentage of cross-reactions between the antigens made from human schistosomes and the antibody produced by the avian schistosome cercariae which cause dermatitis in man. Bird schistosomes are widely distributed and found in areas of human schistosomiasis. Cross reactions, if they existed, would seriously hamper the use of the skin test in suspected cases of the human disease. The results of the present study should be of importance in justifying the use of intradermal antigens for the diagnosis of schistosomiasis in areas where the host may have been exposed to avian schistosome cercariae. One should not generalize, however, that this would be true for all species of non-human schistosome cercariae which cause schistosome dermatitis in man. As previously mentioned, persons exposed to the cercariae of a non-human strain of *S. japonicum* do give positive skin reactions to the antigens made from the human strain of the same parasite. It may be assumed that after the penetration of the cercariae into the human body, the schistosomulae of the non-human strain of *S. japonicum* may show more development than those of an avian schistosome although they do not develop into adult worms. As a result, more antibodies will be produced in the former case than in the latter, leading to a more common occurrence of the phenomena of sensitization. It seems probable, therefore, that the negative results obtained in the present study were due to the low titer of antibody production in the dermatitis cases caused by avian schistosome cercariae.

In the Federated Malay States, an endemic of *sawah* itch, a schistosome dermatitis due to the penetration of the cercariae of a cattle schistosome, *S. spindale*, has been reported (Buckley, 1938). Although there is no evidence that cercariae of *S. spindale* ever develop into adult schistosomes in man, it has been demonstrated that in monkeys (*M. sinicus*) experimentally infected with cercariae of this schistosome, schistosomulae were numerous in the pulmonary, portal, and mesenteric vessels on the 11th day after exposure; and by the 23rd or 24th day most living schistosomulae had disappeared; furthermore, in all the monkeys exposed to infection a strongly positive complement fixation reaction rapidly developed. This became negative within 3 months (Fairley, 1927; Fairley *et al.*, 1930). As this schistosome prevails in certain important areas of human schistosomiasis, it will be interesting to find out whether or not as in the case of infection of the non-human strain of *S. japonicum* in Formosa, persons suffering from *sawah* itch will give positive intradermal reactions to the antigens of human schistosomes.

SUMMARY

Forty-nine recent or old cases of schistosome dermatitis which occurred mainly in the vicinity of Douglas Lake, Michigan, were tested intradermally with the worm antigen of *S. japonicum* and cercarial antigen of *S. mansoni*. At the same time the antigens were tested intradermally on 49 cases that had had no history of schistosome dermatitis; and the results were negative.

It is concluded that schistosome dermatitis cases, caused by the penetration of the cercariae of avian schistosomes, seldom give positive intradermal reactions to the antigens made from the human schistosomes, *S. japonicum* or *S. mansoni*, due probably to a limited antibody production. A differential diagnosis between schistosome dermatitis and seabather's eruption of non-schistosome origin can not be made adequately by an intradermal test with human schistosome antigen. The results of the present study also indicate that the use of intradermal antigens for diagnosis of human schistosomiasis is valid even in the areas where the people may well have been exposed to avian schistosome cercariae.

ACKNOWLEDGMENT

We wish to express our sincere appreciation to Dr. W. W. Cort, Professor of Parasitology, University of Michigan Biological Station, for his interest and help in undertaking this study; to Dr. Lawrence S. Ritchie, Professional Adviser, 406th Medical General Laboratory, U. S. Army, for the preparation of the *S. japonicum* antigen; and to Dr. O. D. Standen, the Wellcome Laboratory of tropical Medicine, London, for the supply of *S. mansoni* antigen.

REFERENCES

Annual Report, 1949. Specificity of *S. japonicum* antigen, *Annual Historical Report, 406th Med. Gen. Lab., % P.M., San Francisco*, pp. 12–13.

AUGUSTINE, D. L., AND WELLER, T. H., 1949. Experimental studies on the specificity of skin tests for the diagnosis of schistosomiasis, *J. Parasit.* **35:** 561–466.

BUCKLEY, J. J. C., 1938. On the dermatitis in Malays caused by the cercariae of *Schistosoma spindale* Montgomery, 1906, *J. Helmin.* **16:** 117–120.

CHU, G. W. T. C., 1952. First report of the presence of a dermatitis-producing marine larval schistosome in Hawaii, *Science* **115:** 151–153.

CORT, W. W., 1928. Schistosome dermatitis. I. Present status of the subject, *Am. J. Hyg.* **23:** 349–371.

CULBERTSON, J. T., AND ROSE, H. M., 1942. Skin tests in schistosomiasis with antigen from *Pneumoneces medioplexus*, *Am. J. Hyg.* **36:** 311–315.

FAIRLEY, N. H., 1927. The early spontaneous cure of bilharziosis (*S. spindalis*) in monkeys (*Macacus sinicus*) and its bearing on species immunity, *Ind. J. Med. Res.* **14:** 685–700.

FAIRLEY, N. H., MACKIE, F. P., AND JASUDASAN, F., 1930. Studies in *Schistosoma spindale*. Part IV. Further observation on spontaneous cure in *Macacus sinicus*, *Ind. Med. Res. Mem.* **17:** 53–59.

FAIRLEY, N. H., AND WILLIAMS, F. E., 1927. A preliminary report on an intradermal reaction in schistosomiasis, *Med. J. Australia* **2:** 811–818.

HASSAN, A., AND EL BETASH, M. H., 1934. *Fasciola gigantica*, an antigen for the skin reaction in human schistosomiasis, *J. Egypt. M. A.* **17:** 991–993.

HSÜ, H. F., AND HSÜ, S. Y. LI, 1956. On the infectivity of the Formosan strain of *Schistosoma japonicum* in *Homo sapiens*, *Am. J. Trop. Med. & Hyg.* **5:** 521–528.

HSÜ, H. F., HSÜ, S. Y. LI, AND RITCHIE, L. S., 1955. Epidemiological study on schistosomiasis japonica in Formosa, *Am. J. Trop. Med. & Hyg.* **4:** 1042–1048.

HUTTON, R. F., 1952. Schistosome cercariae as the probable cause of seabather's eruption, *Bull. Marine Sc. Gulf Caribbean* **2:** 346–359.

KAN, H. C., 1936. Intracutaneous test with *Schistoma japonicum* antigen (a preliminary report), *Chinese Med. J. Suppl.* **1:** 387–393.

KATZIN, B., AND MOST, H., 1946. Cercarial antigen (*S. mansoni*) skin test in diagnosis and management of schistosomiasis japonica, *Bull. U. S. Army Med. Dep't.* **6:** 613–616.

KHALIL, M. B., AND HASSAN, A., 1932. A preliminary note on a new skin reaction in human schistosomiasis, *J. Egyptian M. A.* **15:** 129–130.

MACFARLANE, W. V., 1949. Schistome dermatitis in New Zealand, *Am. J. Hyg.* **50:** 152–167.

PENNER, L. R., 1949. *Cercaria littorinalinae* sp. nov., a dermatitis-producing schistosome larvae from the marine snail, *Littorina planaxis* Philippi, *J. Parasit.* **36:** 466–472.

STRAUSS, J. S., 1956. Seabather's eruption, *Archiv. Dermatol. A.M.A.* **73:** to be published.

STUNKARD, H. W., AND HINCHLIFFE, M. C., 1952. The morphology and life-history of *Microbilharzia variglandis* (Miller and Northrup, 1926) Stunkard and Hinchliffe, 1951, avian blood-flukes whose larvae cause "swimmer's itch" of ocean beaches, *J. Parasit.* **38:** 248–265.

TALIAFERRO, W. H., AND TALIAFERRO, L. G., 1931. Skin reactions in persons infected with *Schistosoma mansoni*, *Porto Rico J. Pub. Health Trop. Med.* **7:** 23–35.

CYTOCHEMICAL STUDIES ON THE HEPATIC TISSUE OF MICE FOLLOWING INFECTIONS WITH *SCHISTOSOMA JAPONICUM*

TOSHISADA SAWADA, KEIZO HARA, KOJI TAKAGI, YASUCHIKA NAGAZAWA AND SUKETAKA OKA

Department of Hygiene, School of Medicine, Gunma University, Maebashi, Japan

Numerous histopathological studies on liver damage following infections with *Schistosoma japonicum* have been reported. The basic features of change are the appearance of a pseudotubercle around the egg and an increase of connective tissue in the portal spaces (Fisher-Rostock, 1930; Belding, 1942; Craig and Faust, 1949; Ito, 1954). Changes in the hepatic parenchyma include atrophy, cellular swelling and coagulative or hyaline necrosis of the cells; however, the extent of damage is usually slight or local (Wakabayashi, 1928; Meleney *et al.*, 1952; 1953). It is generally accepted that these liver changes are caused by toxic products of the adult worms or eggs. Meleney and his associates maintained that areas of "coagulative necrosis" of the hepatic parenchyma might be induced by infarcts resulting from occlusion of the vessels by worms. Furthermore, Takeda *et al.* (1953) demonstrated that granulomas caused by *S. japonicum* are similar to the fundamental form of those induced by allergic pathogens. Some investigators (Ohta and Nishizaki, 1936; Daugherty *et al.*, 1954) have shown striking interference of hepatic function by means of tests for protein or nitrogen metabolism; consequently, the changes in the hepatic parenchyma and connective tissue are important for recognizing and understanding hepatic disease caused by *S. japonicum*. No reports have thus far been made regarding cytological or cytochemical changes.

Our objective is to describe cytochemical changes in the hepatic cells, ground substances of connective tissue, and hemosiderin-like pigment in Kupffer cells of the liver in mice infected with *S. japonicum*. In chronic serous inflammations, acid mucopolysaccharides, which are the main constituents of connective tissue and have an important relationship in allergic diseases, must be given special consideration as an aspect of circulation disturbance.

MATERIALS AND METHODS

Both mature male and female albino mice (14.5–30 gm.) were infected by subcutaneous injection of 80 cercariae. Animals were sacrificed in groups of five after infection intervals of 7, 8 and 9 weeks; these groups will subsequently be referred to as A, B and C respectively. The livers and mesenteric vessels were inspected for the presence of macroscopic lesions and worms. Pieces of liver were fixed in neutral formalin, Carnoy's fluid, and Kolster's solution. After paraffinization, the blocks were sectioned at 4–5 microns. Sections fixed in formalin were stained with hemotoxylin and eosin, Bielschowsky's silver-impregnation method, Weigert-Van Gieson's and Mallory's stains (azocarmine, analin blue and orange G) for connective tissue. Carnoy-fixed sections were

TABLE 1

Histochemical tests applied to hemosiderin-like pigment

Reagent	Method	Fixative	Result
Dye affinity tests			
Eosin, 0.5% (Grubler's)		Neutral formalin	–
Safranine, 1% (Gr.)		N.F.	–
Iron haematoxylin (Gr.)	Heidenhain's	Kolster's	–
Haematoxylin, 0.1% (Gr.)		N. F.	–
Toluidine blue, 0.05% (Gr.)	Ohno's	Carnoy	–
Basic fuchsin	Zeil's	N.F.	–
Methyl green-pyronin (Gr.)	Unna-Pappenheim's	Carnoy	–
Iron tests			
Prussian blue test for ferric iron	Perls'	N. F.	–
Turnbull's blue test for ferrous iron	Tirmann and Schmeltzer's	N. F.	–
Elimination for masked iron	Macallum's	Carnoy, N. F.	–
Microincineration		Carnoy, N. F.	–
Organic and inorganic solubility tests			
Water	72 hours (18°C); 1 hour (100°C)	N. F.	–
Alcohol	10–100%	N. F.	–
Xylol	24 hours	N. F.	–
Chloroform	24 hours	Carnoy, N. F.	–
Glycerin	72 hours	N. F.	–
Aqua ammoniac	12 hours	Carnoy, N. F.	–
Hydrochloric acid (dilute)	12 hours	Carnoy, N. F.	–
Alcohol sulfate (40%)	24 hours	Carnoy, N. F.	+
Alkaline alcohol (70%)	3 hours	N. F.	–
Ethylether	24 hours	Kolster	–
Cold acetone	24 hours	Kolster	–
Sulphuric acid 35N	24 hours	Kolster	–
Decolorization by oxidizing agents			
Potassium permanganate	Pal's	Carnoy, Kolster, N.F.	+
Chlorine	Mayer's	Carnoy, N. F.	+
Bromine	Hawas's	Carnoy, N. F.	+
10% Hydrogen peroxide	24 hours	Carnoy, N. F.	–
Fat stains			
Sudan III (Gr.)		N. F.	–
Sudan black B (Gr.)	Feldman's	N. F.	–
Nile blue sulfate (Gr.)	Smith's	N. F.	–

TABLE 1—*Continued*

Reagent	Method	Fixative	Result
	Bile pigment tests		
Gmelin reagent	Gmelin's	Carnoy, N. F.	–
Iodine reagent	Stein's	Carnoy, N. F.	–
	Fluorescence tests		
Primary fluorescence		Carnoy, N. F.	–
Secondary fluorescence	Borst and Königsdörffer's	Carnoy, N. F.	–
	Caustic silver tests		
Silver impregnation	Bielschowsky's	N. F.	+
Argent-affinity	Masson's	N. F.	+
Argent-affinity	1% caustic silver in dark room		±
	Other tests		
Pseudoperoxidase R.	Lison's	Formalin-lead sol.	–
PAS	Ito's	Carnoy	–
Colloidal iron	Rinehart's	Carnoy	–
Digestion with saliva	3 hours, 37°C	Carnoy, N. F.	–

treated with the periodic acid-Schiff (PAS) technique of Ito *et al.* (1951) for the demonstration of glycogen and other substances positive by this procedure. Control sections were incubated in a saliva solution prior to staining. Desoxyribonucleic acid (DNA) and ribonucleic acid (RNA) were demonstrated by the Feulgen reaction and Bracket's methyl-green pyronin stain, with and without enzymic digestion. Frozen sections of 14 microns from formalin-fixed blocks were stained for lipids by means of Sudan III. In order to demonstrate hyaluronic acid of acid mucopolysaccharides in connective tissues, sections were stained by Ohno's method (0.05 per cent toluidin-blue) and Reinhart's colloidal iron stain, both with and without pre-treatment by bovine testicular hyaluronidase with 2000 VUM per mg. The interpretation of these staining procedures has been discussed in previous papers (Hara *et al.*, 1954 and 1955). Kolster-fixed sections were stained with Heidenhain's iron-hemotoxylin for the demonstration of mitochondria. As a means of demonstrating cellular atrophy or hypertrophy of the Kupffer cells, 100 cells of appropriate sections were measured by means of a filar micrometer. Five control mice (2 male, 3 female, averaging 20 gm. each) were studied by means of the same procedures. Detailed observations for hemosiderin-like pigments, which were deposited in Kupffer cells or histiocytes, were made on selected sections. As shown in Table 1, various tests for iron and bile

pigments were made; and the effect of oxidizing agents and pseudoperoxidase, solubilities with organic and inorganic agents, and affinities of several dyes and silver on certain histopathologic elements were investigated.

RESULTS

Macroscopic Findings

From 7–9 weeks after the schistosome infection was established, considerable loss of body weight had occurred (average 3.5 grams), and a definite decrease in liver size was noted in all cases. All livers of groups A and B (7 and 8 week infection intervals) were dark red in color, hard, and their surfaces were granular (Fig. 1). For group C (9 weeks) they were grayish black in color, harder in texture, and their surfaces markedly roughened by the many projecting miliary nodules which were white with a yellowish tinge. The lobular pattern was obscured in all animals. Many worms were recovered in the mesenteric vessels in all cases.

Cytochemical Observations

General findings. The histopathological picture of the liver was essentially the same as that reported by previous investigators, that is, many eggs with the characteristic pseudotubercles occurred in the portal spaces. Some of the eggs were deposited without cellular reactions, but endothelial swelling and passive congestion of the large veins occurred, and hemosiderin-like pigment was found in the enlarged Kupffer cells and histiocytes. These changes were more prominent in groups B and C. Amorphous eosinophilic materials existed around the shells of the eggs in some animals of group C. Cellular infiltrations of the parenchyma,

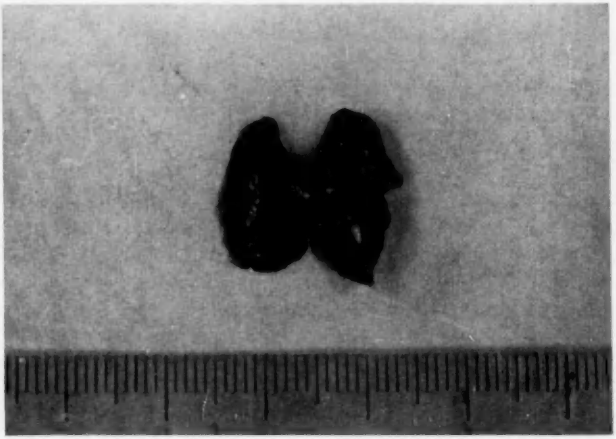


FIG. 1. Photograph of the liver showing a fine nodularity and a diffuse thickening of the capsule.

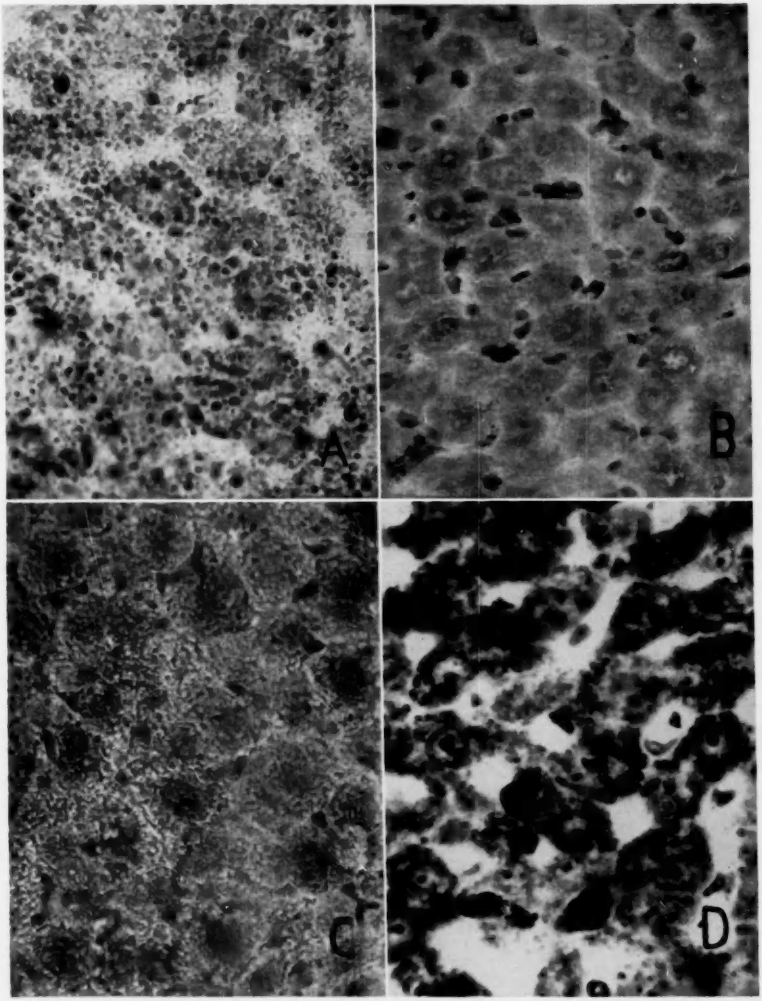


FIG. 2-A. Heavy accumulation of fat droplets in the hepatic cytoplasm. Distribution is marked by the cell border. Sudan III stain; × 640.

FIG. 2-B. General appearance of RNA and DNA in hepatic cells. The liver cells show reduction and homogenous appearance of RNA content, and the nuclei contain little DNA. Kupffer cells are enlarged and rich in hemosiderin-like pigment. Methyl Green-Pyronin stain; × 640.

FIG. 2-C. General appearance of RNA and DNA in the hepatic cells of normal mice. RNA content in cytoplasm is richer than that of exp. animal, and is granular. DNA is abundant in the nuclei. Methyl Green-Pyronin stain; × 640.

FIG. 2-D. Mitochondria showing small or enlarged shape and irregular distribution in the hepatic cytoplasm at the periphery of lobule. Iron-hematoxylin stain; × 1200.

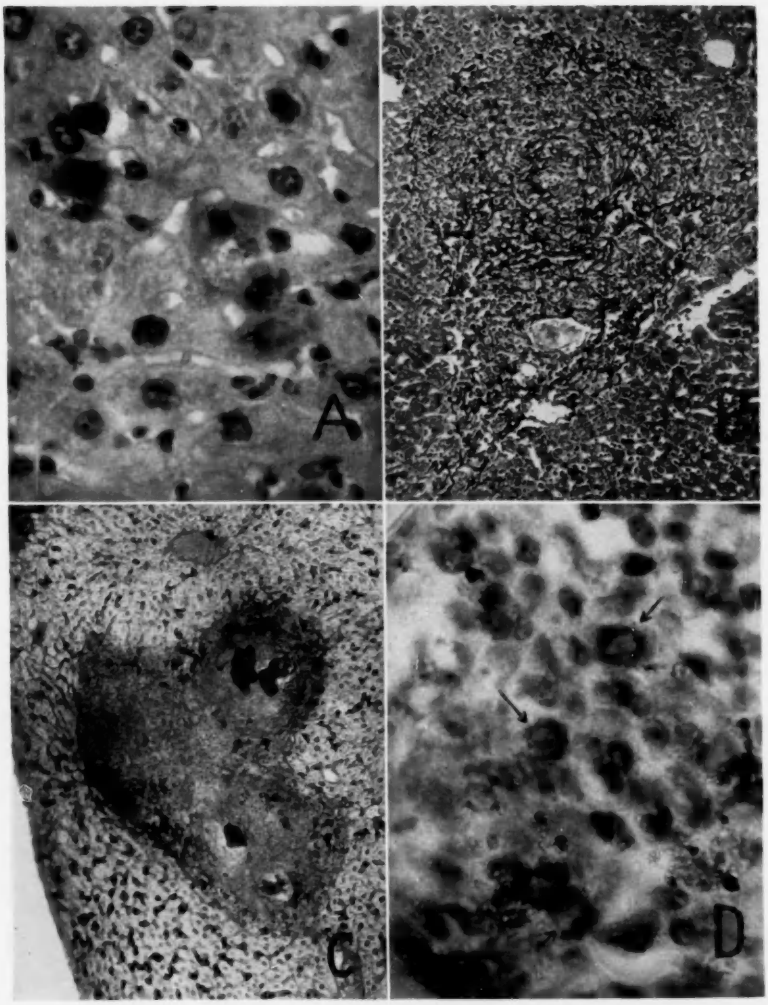


FIG. 3-A. General appearance of hepatic nuclei showing the various degrees of pycnosis, or karyorrhexis. The hepatic cytoplasm shows many fine vacuoles and reduction of cytoplasmic basophilia. Hematoxylin-eosin stain; × 640.

FIG. 3-B. Egg lesion showing a remarkable increase of connective tissue. Bielschowsky's stain; × 200.

FIG. 3-C. PAS-positive material in egg lesion, which was not digested with saliva. Note surrounding hepatic cells showing a complete loss of PAS-positive material with saliva digestion. Periodic acid-schiff stain, × 200.

FIG. 3-D. Pyroninophilic cells showing a large, oval and light nucleus. Methyl Green-Pyronin stain; × 640.

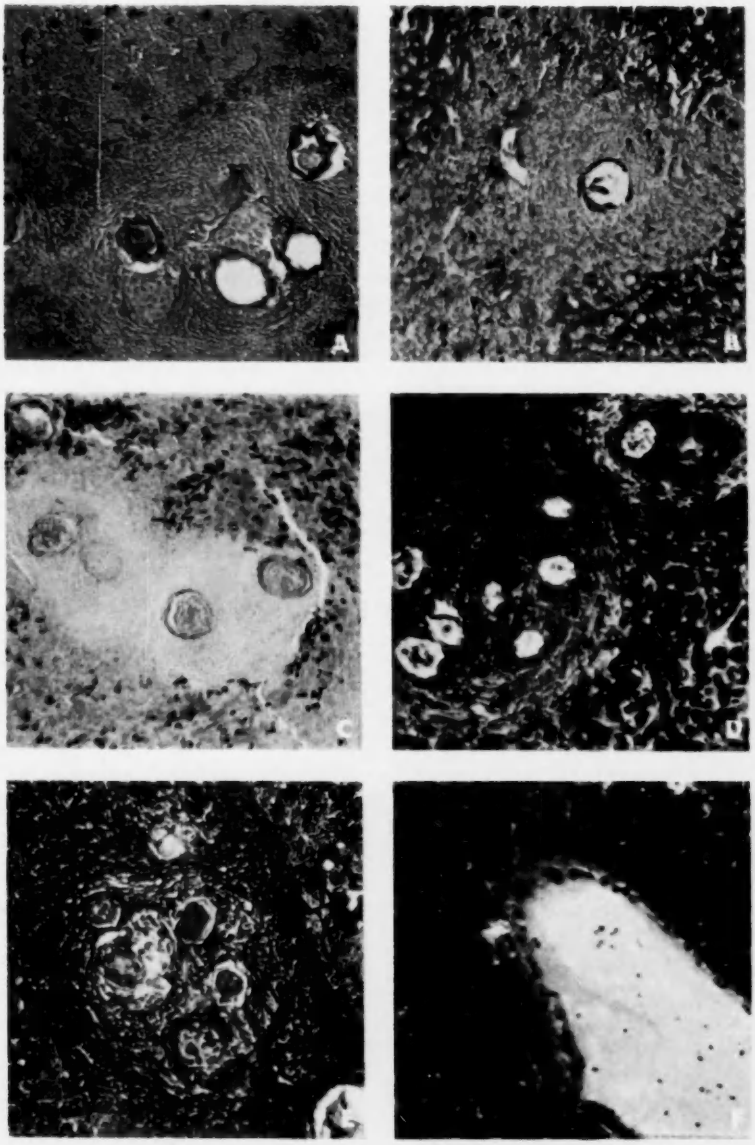


Fig. 4.-A, a mucinous ground material in the egg lesion, which shows green color. Rinehart's stain ; × 200.

Fig. 4.-B, PAS-positive material in the egg lesion. The heavy accumulation of glycogen is evident in the surrounding hepatic cells. Periodic acid-shiff stain ; × 200.

Fig. 4.-C, egg lesion showing marked reduction of mucinous ground material after hyaluronidase digestion. Rinehart's stain ; × 200.

Fig. 4.-D, metachromatic material in the egg lesion, which shows a red purple color. Toluidine blue ; × 200.

Fig. 4.-E, same lesion as shown in D. The remarkable increase of connective tissues fibers is noticed. Mallory's stain ; × 200.

Fig. 4.-F, moderate accumulation of metachromatic material in the media of portal vessel. Toluidine blue ; × 200.

Made in Japan

and coagulative necrosis of liver cells about the central vein and the portal spaces could be found in some animals.

Observations on parenchymal cells. Hypertrophy or atrophy of cells was not prominent in most cases. The cytoplasm of mesenchymal cells contained many fine vacuoles (Fig. 3A). Cytoplasmic basophilia exhibited an irregular distribution, as indicated by decreased staining with hemotoxylin and eosin. Neutral fat content, occurring in the form of fairly large droplets, was moderately increased in all lobes and generally distributed at the periphery of the hepatic cells (Fig. 2A). There was no consistent difference in fat distribution between central and peripheral areas of a lobule. In the liver lobules of control animals stainable lipids did not occur, except for an occasional cell. Glycogen content was higher in the experimental animals as compared with the controls (Fig. 4B), and it was uniformly distributed throughout all lobules.

The RNA content of the cytoplasm in the parenchymal cells was generally less than that of the control, and its distribution gave a homogenous appearance, whereas in the control it took the form of irregular, markedly pyroninophilic granules (Fig. 2B, 2C). Throughout the lobules of the experimental animal the RNA distribution was irregular, while in the controls it was uniformly distributed.

Mitochondria appeared in various forms throughout the lobules. They took the form of fine granules, spherical balls or a vesicular mass. They were distributed irregularly in the cytoplasm and varied in number (Fig. 2D). In some cases of group B mixed forms of small and large granules occurred, while rod-like mitochondria were seen in only one animal of group A. There was no localization of a definite type within a lobule. In the control animals, on the other hand, the mitochondria appeared as small granules in the central region of the lobules, as larger granules in the middle zone and as balls in the peripheral area. Their distribution was regularly uniform within the cytoplasm. Mitochondria of the filamentous type were not seen in either experimental or control animals.

Observations of the parenchymal nuclei. Pycnosis, karyorrhexis and shrinkage were noted in the hepatic parenchyma from all sections of groups A, B and C material. There was no frequency of occurrence of pycnosis and shrunken nuclei were highest in group C (Fig. 3A). No such changes were observed in sections from control mice. The pycnotic nuclei were smaller, denser and irregular in form, and the chromatin showed strong basophilia. When karyorrhexis occurred, the nuclei showed a striking decrease in basophilia, and there was a fragmentation of the chromatin. The shrunken nuclei constituted a more degraded form of the karyorrhectic condition. They were irregular and denser in form due to a condensation of the chromatin; actually, the decrease in volume was not excessive. For all groups karyorrhexis was the most common change, and the definite correlation between these conditions and the presence of eggs. There was no evidence of glycogen content in three degraded cells. The DNA content was most intense in the pycnotic and shrunken nuclei, where karyorrhexis occurred. There was less reaction for total DNA content than in the other degraded nuclei. The reaction for RNA in the cytoplasm of these cells, however, was not reduced to the same degree as that for DNA in the nucleus.

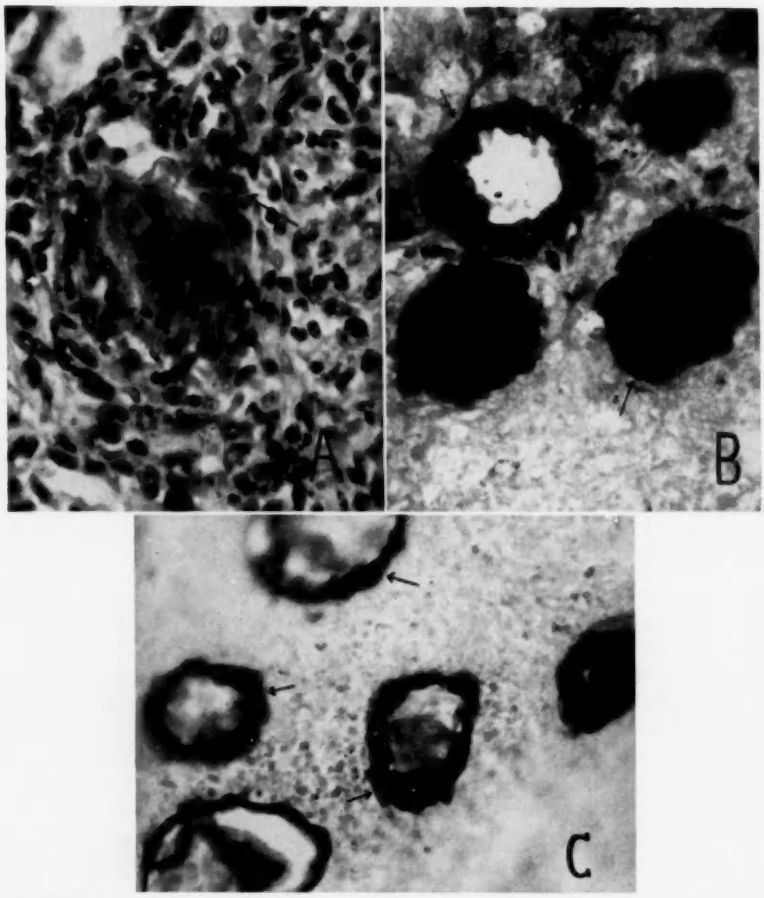


FIG. 5-A. Flame-like, red-staining material about egg with a tendency to extend outward from the eggshell. Hematoxylin-eosin stain; × 680.

FIG. 5-B. Flame-like substances about eggshell showing PAS-positive character. Periodic acid-schiff stain; × 680.

FIG. 5-C. Flame-like material about eggshell which still retained PAS-positive character after saliva digestion. This material shows the same chemical nature as eggshell. Saliva test, PAS stain; × 680.

Observations of the portal space. Tissue responses to the presence of eggs varied from merely cellular infiltration to the formation of the characteristic pseudotubercles. Associated with these conditions there was a proliferation of connective tissues of increased density (Fig. 3B). PAS-positive materials, which remained even after digestion with saliva, were noticed in the connective tissue

(Fig. 4B, 3C) and in the walls of some portal vessels. They were dispersed between connective tissue cells and fibers. When treated with colloidal iron, they stained green (Fig. 4A). After hyaluronidase treatment, these PAS- and colloidal iron-positive substances were digested (Fig. 4C) and showed metachromasia of a rose-purple color with 0.05 per cent toluidin blue at a pH of 4.1 and 7.0 (Fig. 4D, 4E, 4F), but not at a pH of 2.5. In these observations materials in question were concluded to be acid mucopolysaccharides of the hyaluronic acid type. This material was seen in the vessel walls and in the connective tissue about the egg lesions. The portal spaces of control animals were negative for this substance. In some animals a few pyroninophilic cells involving a large, oval and light-staining nucleus appeared in the periphery of the granulomatous lesions (Fig. 3D). A flame-like, red-staining material surrounded the eggs, but was sharply demarcated from surrounding tissue. This amorphous eosinophilic material was PAS-positive and did not react to saliva, hyaluronidase or colloidal iron, so it was assumed to be a polysaccharide other than glycogen (Fig. 5B, 5C). The egg shell appeared to be of the same histochemical nature as the flame-like material surrounding the eggs.

Observations on Kupffer cells. As shown in Table 2, measurements of the Kupffer cells revealed hypertrophy to the extent of 30–50 per cent. In these enlarged cells there was an increase of the amount of the hemosiderin-like pigment (Fig. 2B). Associated with this increase was a change of its distribution. These changes were particularly associated with animal groups B and C. A small amount of fat in the form of fine droplets was noted. There was no evidence of PAS-positive material. As shown in Table 1, only 40 per cent alcohol sulfate among a number of agents dissolved the hemosiderin-like pigment. Decolorization occurred with such oxidizing agents as potassium permanganate (Pal's method), chlorine (Mayer's) and bromine (Hawa's). A very specific picture was obtained by the reaction with caustic silver. The pigment appeared intensely black in color following Masson's argent affinity test and Bielschowsky's silver impregnation test. No additional information was obtained with other tests for ferric, ferrous and masked iron.

TABLE 2

Frequency distribution of length of Kupffer cells in 15 animals, 100 cells measured from each animal

Length* interval	Experimental mice																Control mice†
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	Mean	
microns																	
4.0–5.9	0	0	0	2	16	18	10	2	0	0	2	0	6	12	2	5	24
6.0–7.9	28	22	22	22	20	8	24	36	46	50	42	28	36	62	62	34	64
8.0–9.9	36	32	38	50	34	38	42	36	42	18	34	52	50	18	28	37	10
10.0–11.9	32	34	38	26	30	34	24	26	10	32	22	14	6	8	8	23	2
12.0–13.9	4	12	2	0	0	2	0	0	2	0	0	6	2	0	0	2	0

* Width of cells was essentially constant for all length intervals.

† Mean of four animals.

DISCUSSION

As a result of these investigations, consistent changes in nuclear morphology have been noted in association with changes in the amount and distribution of certain histochemical constituents of the cell, which may ultimately prove beneficial in evaluating the functional state of the liver under the influence of schistosomiasis infections. Some investigators (Menten and Carpenter, 1951; Akiyama and Nakamura, 1935; Szanto and Popper, 1951; Morishima and Matsuyama, 1954) have maintained that the increase of glycogen content and the decrease of RNA in the cytoplasm of hepatic cells are of histologic significance in judging abnormal function. Yokoyama *et al.* (1953) reported an increased cytoplasmic content of RNA in regnerating mice liver. The increased accumulation of fat droplets was designated (Abell and Beveridge, 1950; Cowdry, 1950) as a sign of degeneration in the prenecrotic phase of the sequence of histologic changes. The DNA content of the nucleus is well known to be associated with cell activity. The present study revealed a marked increase of glycogen and fat, and a decrease of RNA in the cytoplasm of the hepatic cells of infected animals. In association with these conditions karyorrhexis was always conspicuous. In this nuclear condition there was a slight diminution of DNA. The mitochondria of the hepatic cytoplasm showed variable changes in number, form, size and distribution. These changes may, of course, depend to some extent on dietary components, status of digestion and postmortem changes, but seemingly they reflect retrograde processes in cell metabolism (Cowdry, 1916; Roberts, 1949). From these observations we tentatively conclude that the hepatic parenchyma is critically affected by infections with *S. japonicum*.

In contrast to the control animals, there was an increased amount of acid mucopolysaccharides in animals infected with *S. japonicum*. This was in association with an increase in the amount of connective tissue and intercellular substances, and a thickening of the blood vessel walls. In an interpretation of conditions associated with serous inflammation, Rossle (1943) and Eppinger (1949) maintained that the accumulation of protein-rich fluids in the connective tissue probably reflected impairment in the diffusion of metabolic materials to and from the capillary and parenchyma. Altshuler and Angevin (1949) supported this interpretation and also demonstrated the presence of acid mucopolysaccharides in the protein-rich fluids. More recently Altshuler and Angevin (1951) reported that acid mucopolysaccharides generally occur in all cases of serous inflammation. Their occurrence is widely observed in allergic lesions, sclerosis of blood vessels, fibrinoid formations and hormonal imbalance. In our experimental animals it was observed that serous inflammation had been induced by the toxic or mechanical stimulation of the schistosome eggs. The mucopolysaccharides were present only at the egg lesions with the proliferation of connective tissues, and it is suggested that their presence may be linked with the formation of collagen and elastic fibers. Whether the increase of acid-mucopolysaccharides is directly caused by definite etiologic agents is uncertain, but it is associated with hepatic damage in the disease observed here.

The presence of the eosinophilic material deposited around the egg in flame-like radiations suggests a reaction between secretions from the egg and the

tissue fluids (Hoeppli, 1932). This might constitute a specific antigen antibody reaction (Koppisch, 1941) such as is seen when schistosome eggs are placed in serum of infected persons (Oliver-González, 1954). This circumoval material gave the same staining reactions as the egg shell. It was PAS-positive and resistant to saliva digestion. It may possibly contain polysaccharides and constitute some parts of the egg shell. Recently Takeda *et al.* (1953) reported that the granulomas associated with schistosome infections are fundamentally the same as those of allergic origin. Enlargement of Kupffer cells, commonly believed to result from pigment accumulation, must also be interpreted as an allergic phenomenon if we consider the work of Fujimaki (1949) who presented the swelling of Kupffer cells as an allergic phenomenon. The histochemical nature of the pigment of these cells and the histiocytes was studied. It is commonly referred to by many investigators (Fischer-Rostock, 1930; Belding, 1942; Meleney *et al.*, 1952; 1953; Bracken *et al.*, 1948; Lagrange and Scheecqmans, 1951) as hematin or hemosiderin. If it is a product of hemoglobin decomposition, it should give positive tests with pseudoperoxidase and for iron and bile pigment; an affinity should occur with eosin, safranin and iron-hematoxylin stain, but not with silver; it should be soluble with organic solvents; and decolorization tests with oxidizing agents should be negative. The pigment is evidently fixed with Carnoy's solution and formalin, and is positive for silver affinity tests; thus, it is dissimilar to malaria and formalin pigments. It is differentiated from hemofuscin, which commonly occurs in liver hemochromatosis, on the basis of affinity with fat stains. It is dissolved in 40 per cent alcohol sulfate and decolorizes with potassium permanganate, chlorine and bromine, which is contradictory for hemosiderin-like pigments. The most impressive features of our results were the marked affinity with silver and the lack of evidence for the presence of iron. Among many tests, Masson's silver test and microincineration for iron are the most specific for differentiating melanin and hemoglobin deriva-

TABLE 3

Comparison of hemosiderin-like pigment with other pigments detectable in histochemical tests

(Summary of the literature)

Pigments	Tests												
	Basic dyes	Acidic dyes	Sudan III	Caustic silver	Iron tests	Gmelin reaction	Iodine reaction	Fluorescence	Oxidiz. agent	Acid sol.	Alk. sol.	40% Alc. sulfate	Pseudoperoxidase
Hemosiderin-like Pigment	−	−	−	+	−	−	−	−	+	−	−	+	−
Hemoglobin	+	+			+								+
Hemosiderin	−	+	−	−	+		−	−	−	+	−		
Hematoidin	−	+	−	−	−	+	−	−	±	−	+		
Malaria pigment			−	−	+			−	±	−	+	+	
Formalin pigment			−	−	−						+	−	
Hemofuscine	+		+		−								
Porphyrin								+					
Melanin	−	−	−	+	−	−	−	−	+	−	+	−	
Lipofuscin	+		+	±	−			+	−	−	−	−	

tives. The pigment with which we were concerned appears to be very similar to melanin, but one contradiction was its solubility with inorganic solvents. More precise study is necessary on the nature of this pigment. (See Table 3 for comparison of tests for various pigments *in vivo*.)

SUMMARY

Cytochemical studies on the hepatic lesions of DD mice infected with *Schistosoma japonicum* gave additional evidences of hepatic damage, in which significant findings were a marked increase of glycogen and fat, and a decrease of RNA in the cytoplasm of hepatic cells together with the frequent occurrence of nuclear karyorrhexis. In addition, there was the excessive accumulation of acid mucopolysaccharides (hyaluronic acid type) in the connective tissue of schistosomal granuloma and in the walls of portal vessels. The possible effect of these substances was considered to be the induction of the circulation disturbance in parenchymal tissues rather than the toxic effect of schistosomal worms or eggs. The flame-like, eosinophilic deposits about the schistosomal eggs were composed of polysaccharides other than glycogen and closely related to the substance of the egg shell. The hemosiderin-like pigment deposited in the enlarged, Kupffer cells showed a marked affinity with silver and the lack of evidence for the presence of ferric, ferrous and masked iron. Only one of a number of dissolving agents for the pigment was 40 per cent alcohol sulfate. Considering the results of many cytochemical tests, the best possible suggestion for its nature was its analogy to melanine rather than to products of hemosiderin decomposition.

ACKNOWLEDGMENTS

The authors wish to express their deep appreciation to Dr. L. S. Ritchie, Lt. Col. H. J. Baker (406th Medical General Laboratory) and Dr. S. Kawai (School of Medicine, Gunma University) for their kind advice in the preparation of the manuscript.

This work was published in abstract in conjunction with the annual meeting of the Japanese Society of Parasitologists, *Japan. J. Parasitol.* 4(2): 130, 1955.

REFERENCES

ABELL, M. R., AND BEVERIDGE, J. M. R., 1950. Hepatic necrosis induced by dietary means. II. Biochemical changes occurring in the liver during the development of necrosis, *Arch. Path.* **50:** 23–35.

AKIYAMA, S., AND NAKAMURA, K., 1935. A contribution to studies of glycogen contents in the liver, *Tr. Soc. Path. Jap.* **25:** 466–470.

ALTSHULER, C. H., AND ANGEVINE, D. M., 1949. Histochemical studies on the pathogenesis of fibrinoid, *Am. J. Path.* **25:** 1061–1077.

ALTSHULER, C. H., AND ANGEVINE, D. M., 1951. Acid mucopolysaccharide in degenerative disease of connective tissue, with special reference to serous inflammation, *Am. J. Path.* **27:** 141–156.

BELDING, D. L., 1942. *Clinical parasitology*, 3rd ed., Appleton-Century-Crofts, Inc., New York, 570–572.

BRACKEN, M., BAILEY, W., AND THOMAS, H. M., JR., 1948. The lesions of schistosomiasis japonica, *Am. J. Path.* **24:** 611–623.

COWDRY, E. V., 1916. The general functional significance of mitochondria, *Am. J. Anat.* **19:** 423–446.

COWDRY, E. V., 1950. *A textbook of histology*, 4th ed., Henry Kimpton, London, 332–347.

CRAIG, C. F., AND FAUST, E. C., 1949. *Clinical parasitology*, 4th ed., Lea and Febiger, Phila., 430–432.

DAUGHERTY, J., GARSON, S., AND HEYNEMAN, D., 1954. The effect of *Schistosoma mansoni* infections on liver function in mice. I. Amino acid oxidase and ammonia, *Am. J. Trop. Med. and Hyg.* **3:** 511–517.

EPPINGER, H., 1949. *Die permeabilitaets-pathologie*. Julius Springer, Wien, 755 pp.

FISCHER-ROSTOCK, W., 1930. *Handbuch der speziellen pathologischen Anatomie und Histologie*, Fünfter Band, Henke und Lubarsch, Berlin, 709–713.

FUJIMAKI, S., 1949. Immunological significance of hypertrophy of Kupffer's cells of liver, *Tr. Soc. Path. Jap.* **38:** 214–215.

HARA, K., OKA, S., SAWADA, T., AND FUSE, M., 1954. Cyto-chemical observation on *Entamoeba histolytica*, *Gunma J. Med. Sci.* **3:** 249–265.

HARA, K., SAWADA, T., OKA, S., AND TAKAGI, K., 1955. The relationship between cytoplasmic basophilia and the nucleic acid content of *Entamoeba histolytica*, *Gunma J. Med. Sci.* **4:** 67–78.

HOEPPLI, R., 1932. Histological observations in experimental schistosomiasis japonica, *Chinese Med. J.* **46:** 1179–1186.

ITO, T., YOKOCHI, C., AND TSUKAGOSHI, N., 1951. Über den histochemischen Nachweis von Polysaccharid (Glykogen) und Schleim mittels der Feulgen-Bauer'schen Methode sowie der Überjodsäure-Schiff'schen reaktion, *Kitakanto Med. J.* **1-2:** 1–5.

ITO, J., 1954. Studies on host-specificity of *Schistosoma japonicum*. 3. A comparative pathology of different laboratory mammalian hosts infected with *S. japonicum*, *Nissin Igaku* **41:** 258–266. (In Japanese)

KOPPISCH, E., 1941. Studies on schistosomiasis mansoni in Puerto Rico. VI. Morbid anatomy of the disease as found in Puerto Ricans, *Puerto Rico J. Pub. Health and Trop. Med.* **16:** 395–455.

LAGRANGE, E., AND SCHEECQMANS, G., 1951. Experimental *S. mansoni* infection in the cotton rat, *Ann. Parasit. Humaine et Comparée.* **26:** 334–337.

MELENEY, H. E., MOORE, D. V., MOST, H., AND CARNEY, B. H., 1952. The histopathology of experimental schistosomiasis. I. The hepatic lesions in mice infected with *S. mansoni*, *S. japonicum* and *S. haematobium*, *Am. J. Trop. Med. and Hyg.* **1:** 263–285.

MELENEY, H. E., SANDGROUND, J. H., MOORE, D. B., MOST, H., AND CARNEY, B. H., 1953. The histopathology of experimental schistosomiasis. II. Bisexual infections with *S. mansoni*, *S. japonicum*, and *S. haematobium*, *Am. J. Trop. Med. and Hyg.* **2:** 883–913.

MENTEN, M. L., AND CARPENTER, A. M., 1951. Histochemical distribution of glycogen, in kidneys of patients with different pathologic conditions, especially those with lipid nephrosis, *A.M.A. Arch. Path.* **51:** 486–493.

MORISHIMA, M., AND MATSUYAMA, K., 1954. Histochemical studies of nucleic acid of liver cells in various conditions, *Tr. Soc. Path. Jap.* **29:** 525–526.

OHTA, T., AND NISHIZAKI, B., 1936. Experimentelle Untersuchung über den Stickstoffwechsel bei Kaninchenschistosomiasis japonica, *Mitt. Med. Ges. Okayama.* **48:** 442–463.

OLIVER-GONZÁLEZ, J., 1954. Anti-egg precipitins in the serum of humans infected with *Schistosoma mansoni*, *J. Infect. Dis.* **95:** 86–91.

ROBERTS, H. S., 1949. Changes in mitochondrial form, *Anat. Rec.* **104:** 163–183.

RÖSSLE, R., 1943. Über die serösen Entzüdungen der Organe. *Virchows Arch. Path. Anat.* **311:** 252–284.

SZANTO, P. B., AND POPPER, H., 1951. Basophilic cystoplasmic material (Pentose nucleic acid); distribution in normal and abnormal human liver, *A.M.A. Arch. Path.* **51:** 409–422.

TAKEDA, K., ABE, H., NOYA, H., AND TOZAWA, T., 1953. Experimental studies on chronic allergic granuloma and its relation to sarcoidosis, *Tr. Soc. Path. Jap.* **42:** 143–145.

WAKABAYASHI, E., 1928. On the excretion of dye in experimental liver dysfunction with *Schistosoma japonicum* infection, *Exp. Gastroenterology*, **3:** 13–18. (In Japanese)

YOKOYAMA, H. O., TSUBOI, K. K., WILSON, M. E., AND STOWELL, R. E., 1953. Histochemical studies on regenerating mouse liver, *Lab. Invest.* **2:** 91–108.

PULMONARY ARTERIOVENOUS FISTULAS AND ARTERIAL DISTRIBUTION OF EGGS OF *SCHISTOSOMA MANSONI*

J. LOPES DE FARIA

From the Department of Pathology, Faculty of Medicine, University of São Paulo, São Paulo, Brazil

The adult worms of *Schistosoma mansoni* live in the veins of the portal system, mainly in the radicles of the inferior mesenteric vein. The eggs are deposited in the venules of the submucosa in the large bowel and escape through the mucosa into the intestinal lumen, mixing with the feces. However, many eggs do not reach the intestinal lumen but are retained in the intestinal wall itself or may spread to many organs or tissues not related to the portal venous system, i.e., lungs, central nervous system, heart, kidneys, thyroid, etc. (Faust, 1948; Maciel *et al.*, 1954; Lichtenberg, 1955). The eggs reach the lungs through the hemorrhoidal plexus, a communication between the portal and systemic venous systems (Mainzer, 1951). The frequent liver cirrhosis in schistosomiasis widens that communication. The routes of dissemination to other organs are not known and many theories have been adduced. These theories were reviewed by Faust (1948), who admitted that the most probable way is through the vertebral venous system. However, a systemic arterial distribution of the eggs has been reported (Lichtenberg, 1955). This may be through a patent foramen ovale, or through pulmonary arteriovenous fistulas. Faust considered the first eventuality rare.

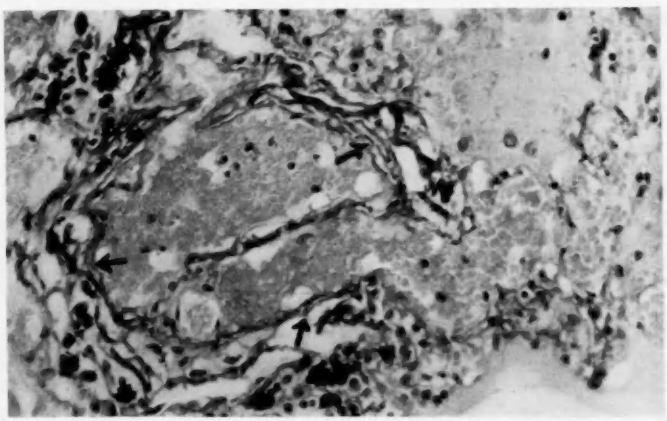


FIG. 1. Pulmonary arteriovenous fistula. Wide arterial lumen divided into two parts by a fibrous tract (old recanalization), the lower one in communication on the right with a venule (50 μ in diameter). (Thin arterial wall, with a single row of muscle fibers between the internal and external elastic membranes—indicated by arrows). Weigert's elastic tissue and van Gieson's stains. ×360.

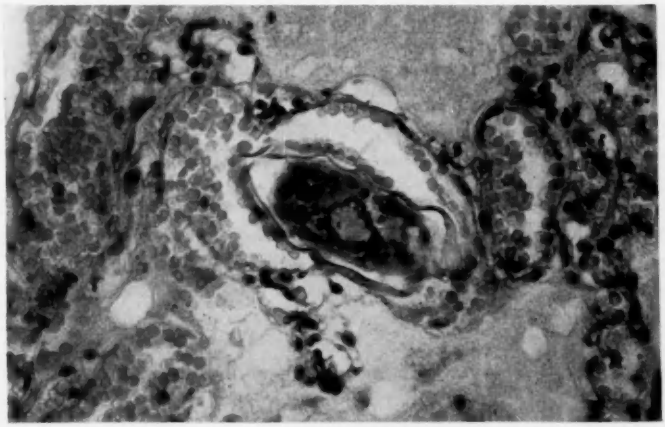


FIG. 2. Schistosome egg in a pulmonary vein. The inferior surface of the egg is adherent to the thin venous wall. Weigert's elastic tissue and van Gieson's stains. ×360.

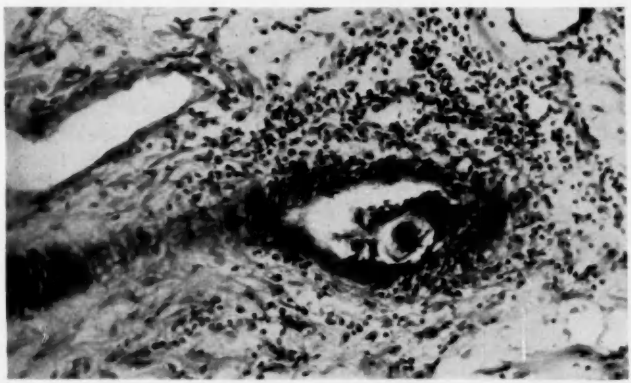


FIG. 3. Large portal space. Schistosome egg in the lumen of a small hepatic artery. Inflammatory reaction in the adventitia and adjacent tissues. Hematxoylin and eosin stain. ×240.

The arteriovenous fistulas were described in one of our previous papers (de Faria, 1954). They were produced by *Schistosoma* eggs which embolized in small pulmonary arteries, causing necrosis of their walls and of adjacent pulmonary veins. There was evidence that the eggs responsible for the necrosis could gain entrance to the pulmonary veins. Through the fistulas additional eggs, arriving in the lungs, might gain entrance to the pulmonary veins and through these vessels to the systemic arterial tree. But this possibility was not emphasized in the former paper. A recent necropsy case of Manson's schistosomiasis (no. 39,412) supports this view.

A twenty-year-old female died of acute diffuse glomerulonephritis. The necropsy disclosed schistosomal rectitis with many eggs, schistosomal liver cirrhosis and a few schistosomal granulomas, as well as not infrequent arteriovenous fistulas caused by the eggs in the lungs (Fig. 1)[1]. There was no evidence of cor pulmonale. In the lung sections a schistosome egg was found in the lumen of a pulmonary vein of 63 μ diameter (Fig. 2). The vessel wall was very thin and consisted of endothelium, an inconspicuous, very thin, elastica interna and an adventitial collagenous layer. A small area of the egg shell was adherent to the venule wall. There was no inflammatory reaction. The schistosome eggs were very infrequent in the liver. In the lumen of a small hepatic artery (125 μ in diameter), however, an egg was found adherent to the arterial wall, covered by a hyaline thrombus and by a few histiocytes. There was necrosis of a few muscle fibers of the artery; the adventitia and periadventitial tissue presented an inflammatory reaction consisting mainly of lymphocytes with many eosinophils and rare histiocytes (Fig. 3).

DISCUSSION

In this case without schistosomal cor pulmonale, a schistosome egg was found in a pulmonary vein and another, possibly carried by the systemic arterial blood, in a small hepatic artery. Possibly the passage of these eggs from the pulmonary arterial tree into the pulmonary vein and into the systemic arterial circulation was through the not infrequent pulmonary arteriovenous fistulas caused by the eggs. Such passage could be accomplished also through the arteriovenous anastomoses found normally in the lungs (von Hayek, 1952). However, the latter anastomoses were not found in the examined lung sections of our case.

CONCLUSION

The arteriovenous fistulas caused by the eggs of *Schistosoma mansoni* in the lungs may constitute a route through which these eggs gain entrance into the systemic arterial system.

REFERENCES

DE FARIA, J. LOPES, 1954. Cor pulmonale in Manson's schistosomiasis. I. Frequency in necropsy material; pulmonary vascular changes caused by schistosome ova, *Am. J. Path.* **30:** 167–193.

FAUST, E. C., 1948. An inquiry into the ectopic lesions in schistosomiasis, *Am. J. Trop. Med.* **28:** 175–199.

v. HAYEK, H., 1952. Die menschliche Lunge und ihre Gefässe, ihr Bau unter besonderer Berücksichtigung der Funktion, *Erg. d. Anat. u. Entwicklungsgesch.* **34:** 144–249.

LICHTENBERG, F., 1955. Lesions of the intrahepatic portal radioles in Manson's schistosomiasis, *Am. J. Path.* **31:** 757–771.

MACIEL, Z., BARROS COELHO, R. AND ABATH, G., 1954. Mielite esquistossomótica (*Schistosoma mansoni*), *An. Fac. Med. Univ. Recife* **14:** 207–219.

MAINZER, FR., 1951. Viscerale Bilharziase (Schistosoma haematobium und mansoni), *Erg. d. inn. Med. u. Kinderheilk.* **2:** 388–411.

[1] Clinical data as well as necropsy and other histopathological findings are to be reported in another paper (in preparation).

THROMBOCYTOPENIC PURPURA DUE TO FUADIN (STIBOPHEN)

JULIO V. RIVERA, HECTOR F. RODRIGUEZ, AND E. PÉREZ-SANTIAGO

The San Patricio Veterans Administration Hospital, San Juan and Bayamón District Hospital, and the School of Medicine, University of Puerto Rico

Fuadin, which is sodium antimony bis-catechol 2,4 disulfonate of sodium, is one of the antimony compounds which has been most extensively used in the treatment of parasitic infections. It is administered by the intramuscular route and is readily absorbed. It is found in largest amounts in the liver, kidneys and thyroid gland (Brown, 1948). In the blood, a larger concentration is found in the erythrocytes than in the plasma (Bahner, 1954; Brown, 1948). It is apparently attached to the globin portion of hemoglobin. About 50 per cent of the antimony is excreted through the kidney at the end of two days. Decreasing amounts may be detected as long as 100 days after injection depending on the dose given (Lippincott *et al.*).

After the usual doses of Fuadin most patients develop muscular pains and electrocardiographic changes (Hernández-Morales, 1945; Lau, 1953). Anorexia and nausea may occur after large doses. An eosinophilia, presumably due to the liberation of antigenic products from the parasite, is not infrequent. Other hematologic effects have been rare.

Mild non-thrombocytopenic purpura after the use of Fuadin was described by Hernández-Morales (1945) in a young girl who showed extensive hepatosplenic disease. Thrombocytopenic purpura occurred in one patient of Hammerschlag (1935) and he referred to similar cases which had ended fatally. Santiago *et al.* (1948) reported one case of bone marrow hypoplasia after the use of Neostibosan and two cases of thrombocytopenic purpura associated with the use of Fuadin.

In view of the rarity of this type of reaction to this much used drug, we are presenting the following case together with experimental observations which may shed some light on the mechanism of its occurrence.

CASE REPORT

J. D., a 33 year old taxi driver who in February, 1952, had been found at this hospital to have schistosomiasis mansoni, was readmitted to San Patricio Veterans Administration Hospital on September 3, 1952, because of bleeding from various sites. Routine laboratory examinations were normal. The patient was discharged with advice to seek treatment at the local public health unit. There he received two courses (40 ml. each) of Fuadin, with a month's interval between, and had noted after each injection of the second series, the appearance of petechiae on his legs and transient blood-streaking of his saliva, which subsided within a day. After another month of rest he was started on a third course of treatment. Following the second and third injections of this series, similar bleeding occurred. The fourth injection was followed by the onset of giddiness,

general malaise and, a few hours later, by the appearance of frankly bloody expectorations, hematochezia, hoarseness and a widespread hemorrhagic eruption. The patient denied having taken any other drugs, or exposure to any potentially toxic materials.

Physical examination revealed a well developed, well nourished young white male who exhibited numerous petechiae and ecchymoses over the whole body. Temperature was 98°F. Extensive sub-conjunctival hemorrhages were present but the retinas were normal. There was marked periorbital swelling and purple discoloration. A mucosanguinous discharge exuded from the nares. Sub-mucosal ecchymoses were present in the oral cavity, fauces, tonsils and vocal cords. The heart and lungs were normal. The liver and spleen were not enlarged and the abdomen was not distended. Neurological examination was normal. No lymphadenopathy was found. Laboratory examinations including urinalysis, leukocyte and erythrocyte counts, serum bilirubin, bromsulphalein test and cephalin cholesterol flocculation were normal. Platelets were 63,000 per cu. mm., bleeding time 18 minutes, coagulation time 4 minutes, prothrombin time 11.8 seconds. Urine urobilinogen was 1.3 Ehrlich units in two hours, fecal urobilinogen 280 units in 24 hours. Electrocardiogram was normal.

A diagnosis of thrombocytopenic purpura induced by Fuadin was made. Corticotropin therapy was begun and a transfusion of fresh blood was given. By September 7, active bleeding appeared to have ceased and the results of laboratory examinations approached normal. On September 10, the patient complained of headache. Nuchal rigidity and a positive Kernig's sign were found. Intracranial hemorrhage was suspected. These findings subsided after two days and no bleeding from other sites occurred after that date. Corticotropin was discontinued on September 22, and the patient was discharged on October 3, 1952.

The patient was again hospitalized from December 2 to December 10, 1952, for the treatment of acute otitis media. Physical examination and routine laboratory examinations, including platelet count, were within normal limits. *S. mansoni* eggs were found on rectal biopsy and stool examination.

On April 21, 1954, he was again hospitalized because of chronic non-bloody diarrhea, occasional tenesmus, easy fatigability and right subcostal pain. His appetite was not impaired and his weight had remained steady. Physical findings were unchanged except for the presence of a slightly enlarged tender liver. Splenomegaly was not detected. Routine laboratory examinations were normal. Stools showed *Strongyloides stercoralis* larvae and viable eggs of *S. mansoni* Bromsulfalein retention was 12% in 45 minutes. Serum albumin was 4.6 gm., globulin was 2.4 gm. per 100 ml. Cephalin-cholesterol flocculation was 3+ in 48 hours. Roentgenologic examinations of the chest, gall bladder and upper gastrointestinal tract were normal.

Treatment with antispasmodics and gentian violet did not result in improvement of symptomatology. In view of this, and under the assumption that the previous reaction had been due to overdosage rather than to drug sensitivity, Fuadin was started in reduced dosage on May 10 (Figure 1). On May 21, a few

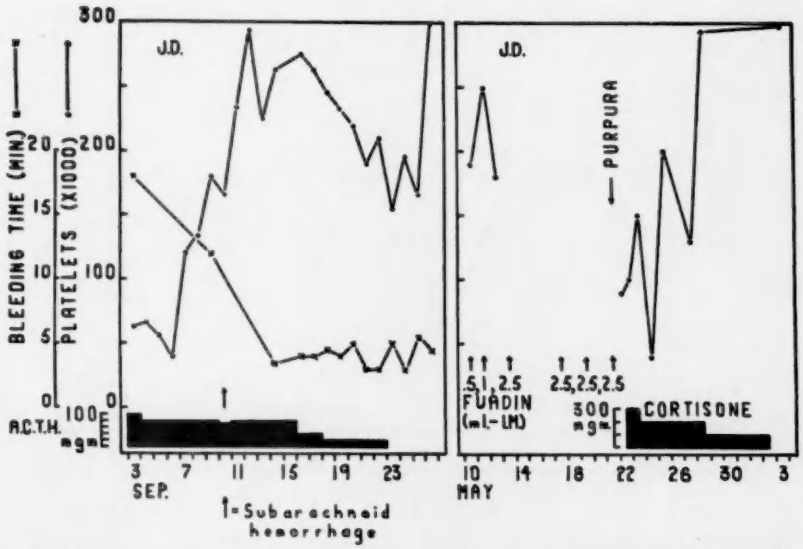


FIG. 1. Platelet levels and bleeding time during first and second episodes of thrombocytopenic purpura.

minutes after the sixth dose (2.5 ml.) the patient suddenly developed headache followed by generalized aching and giddiness. About two hours later there appeared a sparse hemorrhagic eruption on the skin and mucous membranes. An ecchymosis appeared at the site of the injection and at the left elbow. No other swelling or urticaria were noted. Platelets were 90,000 per cu.mm.; bleeding time, 3 minutes; coagulation time, 4½ minutes; prothrombin time, 13 seconds. Clot did not retract in 24 hours. Hemoglobin, leukocyte count, urinalysis and serum bilirubin were unchanged. Fecal urobilinogen was 155 Ehrlich units in 24 hours. Tourniquet test was positive. Electrocardiogram showed depressed T waves in leads V2 through V6. Coombs test was negative.

Therapy with cortisone was started immediately and continued for eleven days. After the second day no further bleeding occurred and the laboratory findings approached normal. Increased capillary fragility was demonstrated till the fifth day after onset of purpura. Liver biopsy on June 15 showed fatty infiltration.

EXPERIMENTAL OBSERVATIONS

In an attempt to demonstrate the presence in this patient's blood of a factor capable of producing thrombocytopenia such as has been found in cases of idiopathic thrombocytopenic purpura (Harrington *et al.*, 1951) and in some cases of drug-induced purpura (Ackroyd, 1953) the following observations were made:

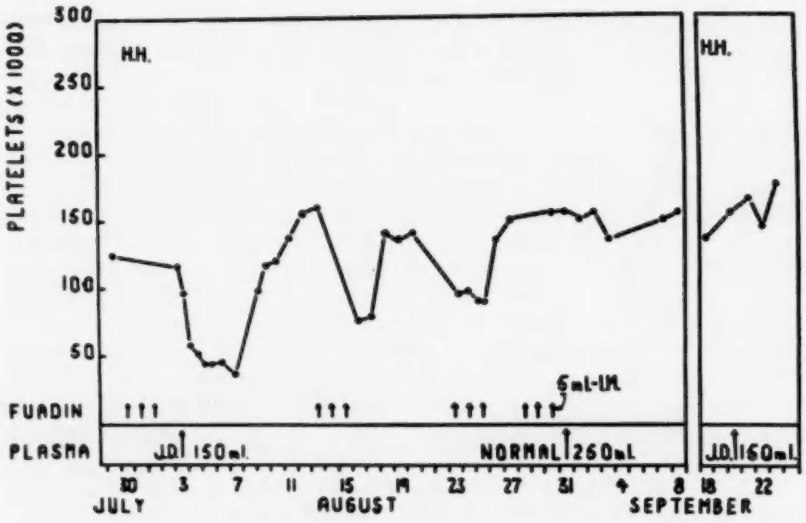


FIG. 2. Platelet levels during experimental administration of J. D.'s and normal plasma and of Fuadin to subject H. H.

A. Three 5-ml. doses of Fuadin were given by the intramuscular route to patient H.H. at Bayamón District Hospital. (Figure 2). This was followed by the intravenous administration of 150 ml. of plasma obtained from patient J.D. on June 23 (33 days after the last episode of purpura). Hematologic studies on patient H.H. were normal before the experiment. Starting two hours after the administration of plasma, a drop in the level of platelets appeared which was maximal four days later. Bone marrow examination at this time showed increased numbers of immature megakaryocytes which showed very infrequent platelet formation. No other significant hematologic change occurred in the recipient. Purpura did not occur.

The platelet level returned to normal by the sixth day. Repeated short courses of Fuadin ten and twenty days after the administration of plasma reproduced the thrombocytopenia. The effect was less marked each time and was not apparent after the twenty-fifth day. To test the specificity of the thrombocytopenic effect, the fourth series of Fuadin was followed by the administration of 250 ml. of commercial pooled plasma. Thrombocytopenia did not result.

B. A second portion of plasma was obtained from patient J.D. on September 10 (112 days after the second episode of purpura). This plasma did not produce thrombocytopenia either in H.H. who was not given Fuadin at this time, or in a third patient, A.R., following three 5-ml. doses of Fuadin intramuscularly. In vitro studies, carried out to demonstrate an anti-platelet substance in the plasma, also gave negative results.[1]

[1] These studies were carried out in Dr. John H. Harris' laboratory, Department of Medicine, Western Reserve University, Cleveland, Ohio.

DISCUSSION

Although various hematologic changes have been observed following the injection of compounds of antimony in experimental animals and human beings, there is no change of sufficient significance to be considered characteristic of antimony poisoning (Lucia and Brown, 1934; Fairhall and Hyslop, 1947). Thrombocytopenic purpura following Fuadin, such as occurred in our patient, has not been described to our knowledge in patients receiving other antimony preparations than Fuadin, therapeutically or during occupational exposure.

Purpura as it occurred in the first case described by Santiago *et al.* (1948) was probably due to high dosage of Fuadin given in the presence of liver disease. Their second case, like the one observed by Hammerschlag (1935) and the one reported here, cannot be explained on this basis because of the small doses of the drug given. An immunological mechanism such as has been demonstrated in certain cases of drug-induced thrombocytopenia (Ackroyd, 1953; Weifuse *et al.*, 1954) seems more probable.

Our experiment appears to demonstrate the presence in the patient's plasma of a substance which is capable of causing thrombocytopenia in the presence of Fuadin. This material was no longer demonstrable 112 days after the patient had received Fuadin for the last time. The fact that thrombocytopenia did not occur immediately upon the administration of Fuadin on the second course may be explained on this basis (insufficient titer of antibody) or by postulating that a minimum amount of the drug is necessary or a minimum time is required for the antigen-antibody reaction to occur or to produce its damaging effect on the platelets, bone marrow or blood vessels. A similar decrease with time of the titer of anti-platelet agglutinins has been described in patients with purpura induced by Sedormid (Ackroyd, 1953) and quinidine (Weifuse *et al.*, 1954), and in a case of immune hemolytic anemia reported by Harris (1954). In quinidine-sensitive patients purpura may not occur until after therapeutic doses have been given for several days (Ackroyd, 1953).

The exact immunological mechanism operating in this patient could not be studied further because of the inability to demonstrate a platelet agglutinin in vitro in the second portion of plasma. (The first portion was unfortunately not so studied). It can only be concluded that a factor appeared in the patient's plasma as a result of exposure to Fuadin which in the presence of the drug is able to agglutinate or lyse platelets, to depress megakaryocyte activity and probably produce capillary damage.

SUMMARY

A case of immune thrombocytopenic purpura due to Fuadin has been reported. The reaction occurred on 2 occasions when the drug was given. Administration of plasma from this patient to a recipient who also received Fuadin produced thrombocytopenia without purpura. It is postulated that thrombocytopenic purpura in this patient was due to the occurrence of a drug-antibody complex capable of destroying or agglutinating platelets and depressing megakaryocyte activity.

ACKNOWLEDGMENT

We are indebted to Dr. John H. Harris for the performance of the in vitro studies and to Dr. Frank Gardner for his valauable advice in the planning of the experimental studies.

REFERENCES

ACKROYD, J. F., 1953. Allergic purpura, including purpura due to foods, drugs and infections, *Am. J. Med.* **14:** 605–32.

BAHNER, C. T., 1954. Localization of antimony in the blood, *Proc. Soc. Exper. Biol. & Med.* **86:** 371–73.

BROWN, H. M., 1948. Treatment of filariasis with anthiomaline, *Ann. N. Y. Acad. Med.* **50:** 51–71.

FAIRHALL, L. T., AND HYSLOP, F., 1947. The toxicology of antimony, *Public Health Reports, Supp. 195.*

GOODWIN, L. G., AND PAGE, J. E., 1943. A study of the excretion of organic antimonials, *Biochem. J.* **37:** 198–209.

HAMMERSCHLAG, E., 1935. A case of Fuadin poisoning, *Wiener Klinische Wochenschrift* **46:** 1431.

HARRINGTON, W. J., MINNICH, V., HOLLINGSWORTH, J. W., AND MOORE, C., 1951. Demonstration of thrombocytopenic factor in blood of patients with thrombocytopenic purpura, *J. Lab. & Clin. Med.* **38:** 1–10.

HARRIS, JOHN W., 1954. Studies on the mechanism of a drug induced hemolytic anemia, *J. Lab. & Clin. Med.* **44:** 809–10.

HASSAN, A., 1938. Distribution of antimony in body organs following administration of therapeutic antimony, *J. Egyptian Med. Assoc.* **21:** 123–25.

HERNÁNDEZ-MORALES, F., 1945. Treatment of schistosomiasis, *P. R. J. Public Health & Trop. Med.* **20:** 322–38.

LAU, F. Y., 1953. Electrocardiographic changes occurring with trivalent antimony compounds during therapy of schistosomiasis, *California M.* **79:** 114–17.

LIPPINCOTT, S. W., ELLERBROOK, L. D., RHEES, M., AND MASON, P., 1947. A study of the distribution and fate of antimony when used as tartar emetic and Fuadin in the treatment of American soldiers with schistosomiasis japonica, *J. Clin. Investigation.* **26:** 370–78.

LUCIA, S. P., AND BROWN, J. W., 1934. The effects of potassium antimonyl tartrate on the blood and hematopoietic organs, *J. Pharmacol. & Exper. Therap.* **52:** 418–29.

SANTIAGO STEVENSON, D., SUAREZ, R. M., AND MARCHAND, E. J., 1948. The use of BAL in heavy metal poisoning, with particular reference to antimonial intoxication, *Puerto Rico J. of Pub. Health & Trop. Med.* **23:** 533–55.

WEIFUSE, L., SPEAR, P. W., AND SASS, M., 1954. Quinidine-induced thrombocytopenic purpura, *Am. J. Med.* **17:** 414–22.

DISTRIBUTION OF *ECHINOCOCCUS* DISEASE IN NORTHWESTERN CANADA

R. W. WOLFGANG[1] AND J. B. POOLE[2]

Echinococcus[3], until recently, has been considered to be a domestic parasite primarily of sheep-raising areas, infecting dogs and man mainly in temperate and subtropical regions (Mackie *et al.*, 1954). It has not been considered of endemic importance in North America and as recently as 1952 Magath wrote "It is evident that of the approximately 650 patients seen in North America. more than 95 per cent have acquired their disease in some country foreign to North America." There is considerable evidence which suggests that even in the United States endogenous infection with *Echinococcus* is possible. Riley (1933, 1939) reported the presence of *Echinococcus* in wolves and moose in Minnesota and discussed the possible importance of a sylvatic cycle. That dogs are found infected in various areas in the United States is borne out by the reports of McClune (1935), Edney (1940) and Franklin and Ward (1953).

Sweatman (1952) and Miller (1953) in Canada, and Rausch and Schiller (1951) in Alaska have reported *Echinococcus* in moose and other deer, foxes, coyotes and wolves. The presence of sylvatic echinococcus in North America seems to be definitely established.

The relationship between sylvatic hydatid and that in man is probably nowhere so important as it is among the Indians and Eskimos in Canada's Northwest because of the role of sled dogs and of wild herbivores in the economy of the natives. The relationship of the adult tapeworms in wolves or the natives' dogs to the larval hydatid stage in herbivores such as moose, elk, caribou and reindeer or to man, is well established.

Miller made a preliminary field study of the hydatid problem in Northwestern Canada in 1953 and further surveys were carried out in the area by Wolfgang in 1954 and Wolfgang and Poole in 1955. At the same time the Institute of Parasitology undertook a study of this parasite with the aim of understanding its epidemiology in wild life and man, together with the development of a satisfactory antigen for determining the presence of hydatid in human beings in Canada.

The antigen used by Miller originated in Australia where it was prepared from sheep hydatid cysts. It has not proved satisfactory in Canadian use. The antigen subsequently prepared at the Institute of Parasitology is made by precipitating

[1] Assistant Professor of Parasitology, Macdonald College, McGill University.

[2] Parasitologist, Laboratory of Hygiene, Department of National Health and Welfare, Ottawa, Canada and Research Associate in Parasitology, Macdonald College, McGill University.

Present address: Sterling-Winthrop Research Institute, Rensselaer, N. Y.

[3] *Echinococcus* refers to *Echinococcus granulosus*. However, the species of *Echinococcus* found in Northwestern Canada is still in doubt. Because of the confusion now existing among taxonomists regarding the northern *Echinococcus* species the authors prefer to use the generic name only until a more accurate description has been established.

particulate matter from fresh reindeer hydatid fluid to which is added a 1:20,000 merthiolate solution. Preliminary tests indicated that patients that were suspected or known to have hydatid cysts, either from X-ray diagnosis or post-operative data, reacted strongly to intradermal doses of about 0.1 to 0.25 ml. of antigen. Both authors used the smaller dose of 0.1 ml. on the 1955 surveys. The reactions were just as rapid as the 0.25 ml. dose but their duration was only about 4 hours as opposed to the 12 to 36 hours for the larger dose.

With this antigen the Institute, in cooperation with the Laboratory of Hygiene and Indian Health Services of the Dominion Department of National Health and Welfare, has conducted a two year testing program of the native population in both the Yukon Territory and the Mackenzie district of the Northwest Territories, thus surveying an area from the 60th. parallel north to the Arctic Ocean.

In 1954, Wolfgang tested 293 Indians in the Yukon district and found 38 per cent strongly reactive to the antigen skin test. In 1955 the same author surveyed the Mackenzie River district along the Mackenzie River from Great Slave Lake to the Arctic Ocean and tested 1,145 natives of whom 41.5 per cent showed a positive reaction. Poole in 1955 surveyed the Great Slave Lake Indian bands and of 584 natives tested, 13.5 per cent gave a positive reaction. The total number of natives tested with Canadian antigen in the two years was 2,022 with 31 per cent giving positive reactions.

The Indian Health Services hospital for this area, located at Edmonton, Alberta, records 180 cases of hydatid throughout the years. Of these 71 were localized in the liver and 109 in the lungs. Thirty per cent of the cases were in the 25-year or under age group; 30 per cent were over 50 years while the remaining 40 per cent were in the 25–50 year group. Miller (1953) lists 141 hydatid cases, some of which are a duplication of the Edmonton records; 36 per cent of these were under 21 years, 53 per cent in the 25 to 70 year group and 11 per cent over 70 years of age. Miller tested 842 natives with the Australian antigen and recorded 15 (1.8%) positive reactions. A part of the Great Slave Lake Indian population tested by Miller in 1953 was retested with Canadian antigen by Poole in 1955. It was possible to compare the results of the two types of antigen in the Fort Rae, Dog Rib band of Indians. Miller tested 229 of these Indians without obtaining a positive reaction. Of the 198 Indians tested by Poole 20 per cent gave positive reactions. A number were substantiated by operation and according to available clinical records no false negative reactions occurred.

Examinations of the wild life cycle of *Echinococcus* and the mode of infection to man in Northwestern Canada reveals an interesting picture. There appears to be no doubt that the main reservoirs in the wild life cycle are the wolf along with the herbivores, moose, elk, caribou and reindeer. Throughout the northwest white trappers and hunters report that hydatid cysts are seen fairly commonly in these animals. Miller (1953) quotes Fuller, the mammalogist stationed at Wood Buffalo Park, as estimating that 5 per cent to 20 per cent of the caribou are infected with *Echinococcus* cysts. Choquette (personal communication) who participated in the annual reindeer kill at Aklavik in 1953, reports that, of 462 animals examined, 28 (6.7%) harbored cysts. Wolfgang estimates the number of

Indian dogs per village on the Mackenzie River at a minimum of 50 with a high of 2,000 at Aklavik. He was able to examine 27 dogs and found 7 (25%) infected. Miller (1953) examined 114 dogs and found 32 (25%) infected.

Another factor which enters the picture is the custom of many Indian bands of capturing wolf pups and raising them along with their own dogs to provide a breeding strain for their sled dogs. The Indians in general allow the viscera of the herbivores to be eaten by their dogs and when this fact is borne in mind, along with the large number of dogs kept in relatively close association with the Indian population, the mode of infection to man is easily seen.

If we accept the figures that have been reported for positive skin test reactions with the Canadian antigen as being a fairly accurate index in regard to the percentage of Indians infected with *Echinococcus* it would appear that in the Yukon district there would be 500 cases of past or present hydatid disease and over 900 in the Mackenzie district. If this is true the Yukon district has almost as many cases, and the Mackenzie district considerably more cases, than have been recorded throughout the years for the entire United States.

SUMMARY

Moose, elk, caribou and reindeer have been recorded with hydatid cysts, and wolves with the adult tapeworm in Northwestern Canada. Fresh reindeer hydatid fluid was used to prepare antigen and used in intradermal tests on Indians in Canada's Northwest. The total number of natives tested with this Canadian antigen in two years was 2,022 with 31 per cent giving positive reactions.

REFERENCES

EDNEY, J. K., 1940. The occurrence of *Echinococcus granulosus* in dogs collected in Murfreesboro, Tenn., *J. Tenn. Acad. Science* **15:** 395.

FRANKLIN, M. A., AND WARD, J. W., 1953. Echinococcus infection in Mississippi. A new record of a natural infection in dogs, *J. Parasitology* **39:** 574.

MACKIE, T. T., HUNTER, G. W., AND WORTH, C. B., 1954. *A Manual of Tropical Medicine.* W. B. Saunders Co. Philadelphia.

MCCLUNE, E. F., 1935. Hydatid disease in Wooler, *J. Am. Vet. Assoc.* **86:** 210–211.

MAGATH, T. B., 1952. Diagnostic tests for hydatid disease based on immunologic phenomena, *Archivos Internacionales Des La Hydatidosis*, 1953, 218–228.

MILLER, M. J., 1953. Hydatid infection in Canada, *Can. Med. Assoc. J.* **68:** 423–434.

RAUSCH, R., AND SCHILLER, E., 1951. Hydatid disease (*Echinococcus*) in Alaska and the importance of rodent intermediate hosts, *Science* **113:** 57.

RILEY, W. A., 1933. Reservoirs of *Echinococcus* in Minnesota, *Minn. Med.* **16:** 744–745.

——— 1939. The maintenance of *Echinococcus* in United States, *J. Am. Vet. Med. Assoc.* **95:** 170–172.

STUDIES ON *ECHINOCOCCUS ALVEOLARIS* (KLEMM, 1883), FROM ST. LAWRENCE ISLAND, ALASKA. III. THE HISTOPATHOLOGY CAUSED BY THE INFECTION OF *E. ALVEOLARIS* IN WHITE MICE[1]

SAROJAM K. MANKAU

Department of Zoology, University of Illinois, Urbana, Illinois and Arctic Aeromedical Laboratory

The name *Echinococcus alveolaris* used in this study is as yet not fully recognized by all zoologists. Leuckart (1863) used the term *multilocular* in reference to the alveolar cystic stage that was reported for man and domestic animals. This designation was also used by Leuckart as well as others before him in a more specific way, such as *Echinococcus multilocularis*. The validity of the name has been more or less in doubt because of the rarity of adequate fertile specimens which appear in man and cattle in widely separated areas over the world. One school of thought argues that the multilocular or alveolar type is only a phase of *Echinococcus granulosus*, whereas the other school believes it to be a distinct species. Klemm (1883) specifically describes the alveolar cyst-producing tapeworm as *Echinococcus alveolaris* and it is his designation that we have chosen to follow.

A good deal of work has been done on the pathology produced by *E. granulosus* in man and in experimental animals, with the major emphasis on the clinical aspects of the disease. The pathology caused by *E. alveolaris* is somewhat different from that due to the former, because of the malignant nature of its growth, ever invading more and more of the host tissue until the death of the host occurs.

Henchen and Bircher (1945) made a somewhat detailed study of the pathology caused by alveolar cysts in the human liver, but due to the consistent failure in the past to produce alveolar cysts in experimental animals, the investigation of the histopathology produced by *E. alveolaris* has been very limited. When white mice were found to be successful intermediate hosts for *E. alveolaris*, a number of mice were infected in the laboratory, and a detailed study of the histopathology caused by this parasite at different stages of infection was undertaken not only in the liver, but also in the lung, kidney, spleen, pancreas and alimentary canal.

METHODS AND MATERIALS

The tapeworms for the infection were obtained by Dr. Lyell J. Thomas from naturally infected foxes of St. Lawrence Island, Alaska.

Ten per cent neutral formalin was used as the fixative for the cytological

[1] Portion of a thesis submitted as partial fulfillment of the requirements for the degree of Doctor of Philosophy, University of Illinois. This work was done under the direction of Dr. Lyell J. Thomas, whose advice and assistance are gratefully acknowledged by the author.

study of the tissues. Formalin was neutralized in order to get good results with azure-eosin methods and for the study of the iron-containing pigments such as hemosiderin and hemofuscin. Zenker's solution, aceto-formalin alcohol, Carnoy's fluid, osmium tetroxide and Bouin's fluid were also used for fixation for special technics. Hemotoxylin and eosin were used as the standard stains for all general purposes. The following stains were used for special technics: picro-indigo carmine, Van Gieson's stain and HCl-orange G method for connective tissue, bilirubin stain, Mallory's hemofuchsin stain, azure A-eosin B method, Van Kossa's method for calcium stain, Best's carmine method for glycogen and Mallory's phloxin method for hyalin stain.

OBSERVATIONS AND RESULTS

The organs that were found to be affected by the alveolar hydatid infection in the present study were liver, lung, spleen, kidney, pancreas and alimentary canal.

Liver. In all cases of infection, the liver was the primary site. Pathological changes could be observed microscopically in this organ a few hours after the ingestion of the eggs. The initial changes produced by the infection can be described as a process of acute inflammation, stimulated by the entrance of the foreign body into the host tissue. The neutrophils first make their appearance in the form of a follicle, often completely masking the microscopic larval vesicle. As the larva increases in size, more and more polymorphonuclear cells are attracted to the focus of the infection. The cells around this follicle undergo necrotic changes. Cloudy swelling is often observed in these cells; the nuclei undergo karyorrhexis and in some cases they become karyolytic. During these necrotic changes, the nuclei of some of the host cells shrink in size and appear hyperchromatic, whereas in other cells they become greatly enlarged, sometimes attaining 2 or 3 times the size of normal nuclei. The cytoplasm becomes granular and often appears hyperchromatic.

In the later stages of the infection, an increasing number of fibroblasts appear in the granulation zone around the larval focus, along with a large number of leukocytes. The fibroblasts undergo excessive proliferation; connective tissue fibers are soon formed and the host tissue undergoes fibrosis. Frequently the blood vessels are extremely dilated accompanied with congestion and hemorrhage. The blood vessel through which the embryo reached the focus of infection is often seen infiltrated with polymorphonuclear cells. In the later stages a large number of mononuclear cells are seen in and around the blood vessels (Fig. 4). The sinusoids become distended, often filled with extravasated erythrocytes and mononuclear cells. The blood vessels also show amyloid infiltration and hyalin degeneration. The larva appears to liberate toxic substances into the encapsulating host tissue which cause a general eosinophilia. Large numbers of eosinophilic cells are seen around the larval foci.

The larva of the alveolar *Echinococcus* continues to grow, invading additional host tissue by successive exogenous and endogenous budding. Soon the entire tissue of the infected organ appears as a mesh of fibrotic adventitia (Fig. 1).

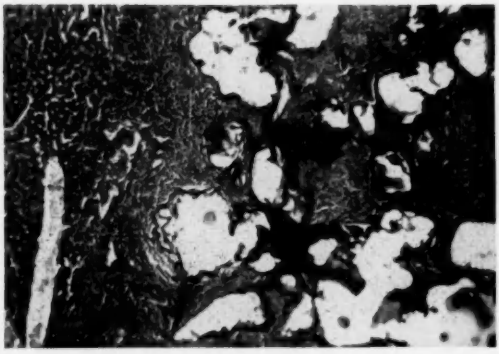


FIG. 1. A group of small, irregular, sterile, alveolar cysts in the liver

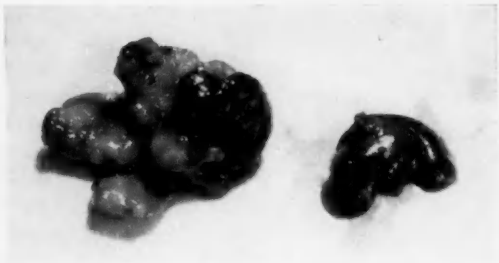


FIG. 2. The liver of a white mouse infected with *E. alveolaris* and the liver of an uninfected mouse. The infected liver is largely replaced by cystic tissue.

The larval tissue eventually replaces the tissue left scattered in the organ as can be seen even in the gross specimen (Fig. 2). At this time a large number of giant cells, especially of Langhan's type, and numerous macrophages are seen removing the ever increasing amount of degenerated host tissue. The excessive fibrosis of the tissue cuts down the blood supply to such areas and consequently the tissue undergoes degeneration, eventually losing its entire cellular structure, and becomes highly eosinophilic. The increasing parasitic tissue exerts a great amount of pressure on the confining host tissue, causing atrophy of the cells around it.

This host reaction at later stages can be described as chronic granulomatous inflammation. The infiltrating mononuclear cells increase in number and are found not only in and around the blood vessels and sinusoids, but also as small groups scattered among the uninfected hepatic cells. In some cases, the gall bladder is also involved by the infection as it becomes surrounded by infiltrating leukocytes and the epithelial cells undergo necrosis.

The bile ducts show a great deal of resistance to necrosis and atrophy, but they undergo excessive proliferation, increasing both in number and size. Occasionally cysts actually develop within the bile ducts. In such cases, the epithelial

cells of the bile ducts become highly columnar and this layer, along with the connective tissue capsule of the bile duct, becomes distended to accommodate the growing parasite within.

Although a great quantity of glycoprotein is used in the formation of the laminated membrane of the alveolar cysts, the amount of glycogen present in the surrounding cells appears normal until they undergo necrosis. In some cases, an increase in the amount of blood pigments such as hemosiderin and hemofuscin is observed, but this is not found to occur consistently in all the animals infected.

Lung. In the experimental infections with white mice, only a single case of pulmonary infection was observed in the 70 odd animals used. The mouse with the pulmonary infection was autopsied six and a half months after the primary infection. Both the liver and lung showed heavy infections, but no other organs were invaded. Two lobes of the lung were completely replaced by the cystic tissue of which a large part was sterile and had undergone atrophy and degen-

FIG. 3. The lung of a mouse infected with *E. alveolaris*

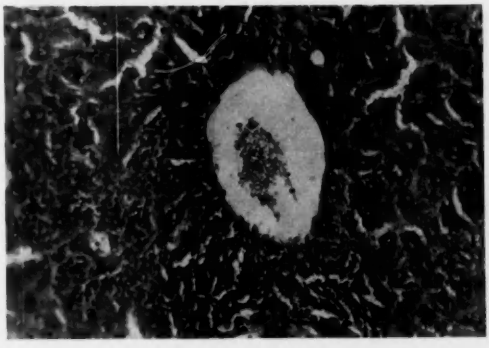


FIG. 4. Infected liver showing perivascular tissue surrounded by mononuclear cells

eration (Fig. 3). A few cysts however, were found to be fertile and they were filled with well developed scolices.

The structure of the cyst itself is very similar to that of the cysts in the liver, although the granulation zone and the connective tissue capsule appear to be narrower than in the hepatic cysts, the former measuring only 25μ–30μ, and the latter 15μ–20μ in thickness. Dew (1928) noted that in the pulmonary infections of *E. granulosus* the laminated membrane is comparatively thin, but in this case the laminated membrane was found to be rather thick, measuring 20μ–25μ as compared to 2μ–10μ in other cysts.

Marked congestion and hemorrhage is observed in the pulmonary tissue. The bronchi and the bronchioles become surrounded by mononucleolar cells (Fig. 5). The bronchial epithelium becomes highly columnar and the goblet cells are very prominent. The bronchi become extremely dilated and the bronchial cavity almost fills with exudate, composed primarily of neutrophils. There is considerable infiltration of mononuclear cells around the blood vessels (Fig. 5). Large numbers of phagocytes and macrophages are present in the alveoli and the phagocytes often contain blood pigment which gives them a yellowish color. Extravasated red blood cells can be seen being removed by macrophages. Subinterstitial inflammation is found to occur in the lung.

This particular case of pulmonary infection was believed to be primary because, first, the lung was rather heavily infected, a few lobes being completely replaced by the cystic tissue. The majority of the cysts were in an advanced stage of atrophy, and some of them were even calcified. The fertile cysts were filled with well developed infective scolices. Second, hepatic cysts in the same mouse had invaded a very large portion of that organ, but were totally sterile and were generally in a stage of degeneration. Therefore, no brood capsules or scolices produced by the liver cysts could have caused a secondary infection in the lung. If the lung infection was secondary, it is of interest that the germinal material from sterile cysts could metastasize and produce fertile cysts in another organ.

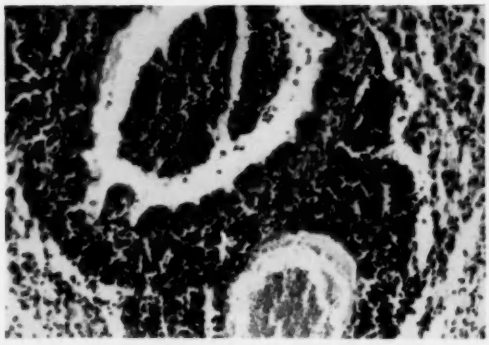


FIG. 5. Infected lung tissue showing perivascular and peribronchial cells surrounded by mononuclears.

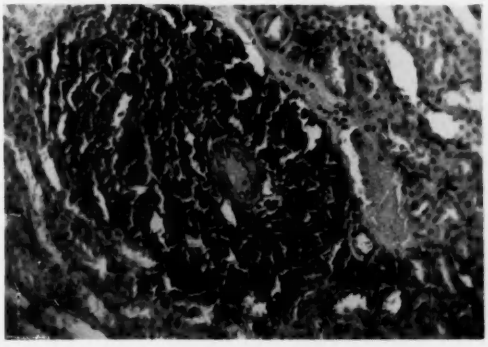


FIG. 6. Perivascular tissue of the kidney infiltrated by mononuclear cells caused by hydatid infection.

Kidney. Hydatid of the kidney is rare, and when present, it occurs by secondary invasion either through metastasis or by implantation. Perhaps this may be due to filtration by hepatic and pulmonary systems which collect most of the embryos, leaving fewer available for peripheral organs and the kidneys. Only two cases of renal infection were observed during this study and both appeared to be secondary infections caused by implantation of cystic tissue from the primary cysts. No cysts were found within renal tissue although it had undergone some pathological change due to hydatids attached to the surface.

Case No. 1. The cyst was enveloped within the perirenal capsule of the kidney. It was ovoid in shape but was found collapsed leaving only a very narrow lumen. The cyst measured only 380 microns in diameter. The hyaline laminated membrane was well developed measuring about 7μ–10μ in thickness. The germinal membrane was quite distinct, but showed no signs of proliferation. The granulation zone was rather narrow and consisted mostly of fibroblasts and a large number of juvenile polymorphs. The perirenal capsule, the ureter and the surrounding fatty tissue were all heavily infiltrated with polymorphs. The capsule was rather thickened and the blood vessels were extremely congested (Fig. 7).

Case No. 2. A large massive cyst was found attached to the kidney and here also it was evident that the infection occurred through implantation rather than metastasis, since the cystic mass was completely outside the renal tissue and there was no apparent attempt on the part of the parasite to infiltrate into the kidney. The cyst was attached to the perirenal connective tissue capsule of the kidney which along with the wall of the cyst, appeared as a single connective tissue capsule.

In structure, the cyst itself was very similar to those found in other organs. The cystic mass was rather large and most of the cysts were found to be highly fertile and filled with scolices, although a few were undergoing degenerative changes characteristic of sterile alveolar cysts.

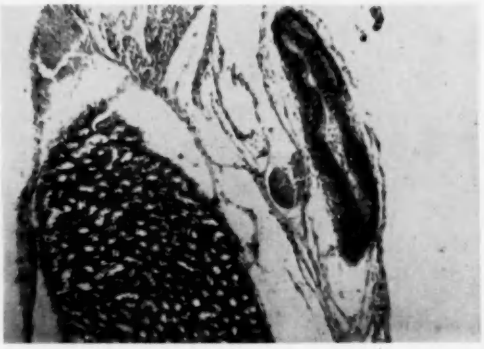


FIG. 7. A small alveolar hydatid cyst attached to the perirenal capsule

While the renal tissue was not affected directly by the cysts, some infiltrative changes occurred within the kidney. There was a considerable amount of perivascular infiltration by mononuclear cells especially in the renal cortex (Fig. 6). Sub-acute interstitial nephritis in the medulla was also produced by the infection.

Spleen. In general, hydatid cysts of the spleen are primary in that they develop from the hexacanth embryo of the parasite. However, no case of primary infection was observed in the author's experimental infections. All the cases of secondary infections must have occurred by means of implantation rather than embolism or metastasis, since in most cases the cysts were attached to the surface of the spleen, and in the few cases where the cysts did occur in the splenic tissue, the major part of the cystic mass was external to the spleen. It was obvious that the cyst had implanted itself on the surface and had sent infiltrating buds into the organ, thus establishing infection within the splenic tissue.

The size of the cystic masses attached to the surface of the spleen varied from 1–4 mm. in diameter. They were all alveolar in type with numerous small vesicles. None of these cysts were fertile. The fatty tissue around the spleen was heavily infiltrated, mainly with lymphocytes, plasma cells and a few polymorphs. The granulation zone was narrow, measuring approximately 17μ thick, with a thin connective tissue capsule. The hyaline or laminated membrane was thin, measuring from 1.5μ–4μ in thickness. Most of the cysts were degenerated and showed signs of calcification (Fig. 8). In cases where the cysts were established within the spleen, a large part of the splenic tissue was replaced by the invading cysts. The structure of the cysts was typical of all the other secondary cysts observed. It is interesting to note that in many of the cysts the laminated membrane was totally absent. The germinal membrane was well developed but no sign of scolex production was observed.

A fairly heavy cellular reaction was observed in the spleen. A definite increase in the number of megakaryocytes and giant cells was observed. Polymorphs and eosinophils were present in large numbers. A great number of red blood cells were found in the sinusoids. There was an increase in the number of myelo-

blasts in the spleen and a slight proliferation of the connective tissue fibers was noticed. Localized fibrosis was also observed in the tissue around the cysts. This may be a repair attempt on the part of the host organ. The white pulp of the spleen was found to undergo excessive proliferation.

Pancreas. Development of hydatid cysts in the pancreas is a very rare occurrence. The only cysts that were found in association with the pancreas in this study were the cysts that developed by implantation. The cysts were small, but there was a great deal of cellular reaction in the surrounding tissues. The cysts showed exogenous and endogenous budding and were always sterile. Large numbers of eosinophils and neutrophils were found around the cysts and they infiltrated the pancreatic tissue causing necrosis. In some cases, complete atrophy of the pancreatic cells was observed. One lobe of the pancreas was entirely enveloped by infiltrating cells.

Alimentary canal. A number of cysts were found attached to the esophagus,

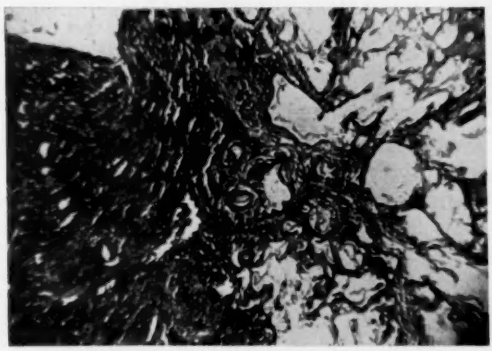


FIG. 8. Sterile alveolar cysts in the spleen, forming additional new foci in the splenic tissue.

FIG. 9. A sterile alveolar cyst attached to the muscles of the alimentary canal. The muscle fibers and the villi appear to have undergone necrosis.

the stomach and the duodenum. The cyst found on the stomach was attached to the muscular wall and was surrounded by fatty tissue heavily infiltrated with leukocytes; it measured approximately 665μ in diameter. The granulation zone measured about 40μ with a thin connective tissue capsule only 17μ–20μ thick. The laminated membrane was also thin, measuring about 1.5μ thick. A great portion of the cyst had undergone complete atrophy. The cellular structure was lost and the cyst was found in an advanced stage of calcification. The cyst showed the typical characteristics of sterile alveolar cysts, with the hyaline membrane, broken and crumpled, lying in the lumen of the cyst (Fig. 9).

There was considerable infiltration of leukocytes within the mucous glands of the esophageal region of the stomach. The area between the tunica propria and lamina muscularis was also infiltrated with leukocytes. The granulation zone of the cyst was found directly above the tunica muscularis. In some parts, the infiltrating leukocytes surrounded the longitudinal muscles and some of them had undergone necrosis and degeneration. Eosinophils, neutrophils, fibroblasts and a few lymphocytes were found in the area of inflammation.

DISCUSSION

Hydatid infections are usually established in the following ways:

1. The embryo that is hatched from the egg in the intestine is carried to the liver or other organs through the blood stream, establishing a focus of infection and developing into a hydatid cyst (Primary infection).

2. Brood capsules, scolices or other germinative parts of the primary hydatid cyst are carried to another site by the lymph or blood stream (Metastasis).

3. Brood capsules, scolices or germinal membrane derived from the intraperitoneal rupture of a primary or a secondary cyst are implanted at new infection sites.

4. Enlargement of the infected liver lobes actually extends them to the other visceral organs and thus establishes the infection by contact.

In the animals used in this study, most of the secondary infections appear to have occurred by means of the second or third methods.

SUMMARY

The liver was found to be the primary site of infection for *Echinococcus alveolaris*; however, lung, kidney, spleen, pancreas and alimentary canal were also found to be affected. The acute cellular reaction caused by the infection and the pathological changes produced in the different organs are also described in detail. Perivascular infiltration with mononuclear cells was found to be characteristic of this infection and was observed in liver, lung and kidney. The chief characteristic of the larval stage of *E. alveolaris* is the type of lesion, which behaves very much like an infiltrating neoplasm.

REFERENCES

Dew, Harold R., 1928. *Hydatid Disease, its Pathology, Diagnosis and Treatment.* Medical Publishing Company of Australia, Sydney.

Henchen, C., and Bircher, R., 1945. Zur Epidemiologie, Pathologie und Chirurgie des *Echinococcus alveolaris*, *Bul. Schweiz. Akad. Med. Wissen.* **1**: 209–224.

MASS TREATMENT OF ASCARIASIS WITH A SINGLE DOSE OF PIPERAZINE CITRATE

F. O. ATCHLEY, D. N. WYSHAM AND E. C. HEMPHILL

Communicable Disease Center, Public Health Service, U. S. Department of Health, Education, and Welfare, West Prestonsburg, Kentucky

Ascariasis is known to affect a large portion of the world's population, and is still highly prevalent in the Appalachian Mountain region of the United States. A safe and practical means of mass therapy would be a very useful public health measure in the control of this disease. Multiple dose therapy is cumbersome and probably unreliable when attempted on a large scale. Consequently, attempts have been made to find a satisfactory method of single dose therapy (Goodwin and Standen, 1954; Hoekenga, 1952). Hexylresorcinol (Crystoids®) has been generally recommended until recently as the drug of choice for treating ascariasis, and is usually given as a single dose. This drug has several disadvantages, including the need for fasting as well as pretreatment and post treatment purgation. Available only in capsules or pills, it is difficult to administer to small children, and there is risk of burning the buccal mucosa. These disadvantages tend to inhibit its use for mass treatment. Hence, a better single dose ascaricide is needed.

CHOICE OF A DRUG

Choice of a drug should be based on ease of administration, cost, and therapeutic index, i.e., the ratio of toxic dose to therapeutic dose. Diethyl-carbamyl-4-methyl piperazine hydrochloride (diethylcarbamazine, Hetrazan) has been used successfully in treating ascariasis, and cure rates with multiple dose treatment over 4–8 days are as high as 80–90 per cent (Oliver-González *et al.*, 1949; Gabbard *et al.*, 1954; Etteldorf and Crawford, 1950; Hoekenga, 1952). When used as a single dose by Hoekenga, however, the cure rate was only 33.3 per cent. This author also noted that an experimental dose of 18 mg. per pound of body weight produced toxic symptoms in all of five children treated. Apparently the therapeutic index is rather low, and there appears to be little likelihood that an effective tolerable single dose can be developed. Hoekenga concluded that diethylcarbamazine "is not an ideal compound for mass treatment because multiple doses are necessary". Its use in mass therapy therefore was not seriously considered.

Santonin and oil of chenopodium were not chosen because of their known toxicity and the lack of evidence that they are more effective than safer drugs (Etteldorf *et al.*, 1950; Hoekenga, 1954; Mackie *et al.*, 1954). The use of hexylresorcinol was considered despite its inconvenience in administration. Belding (1952) had reported on its high efficacy; however, Hoekenga in 1954 revealed that this drug effected a cure in only 42 per cent of 80 patients. Also, its cost per adult dose seemed too high for use in mass therapy.

Several reports have indicated that piperazine citrate is an inexpensive ascarifuge with a high therapeutic index, curative in 90 to 100 per cent of cases given multiple doses over several days. It is commercially prepared in pleasant tasting syrups, readily accepted by small children. No significant toxicity has been reported in its use for ascariasis (Brown, 1954; Brown and Sterman, 1954; Goodwin and Standen, 1954; Swartzwelder *et al.*, 1955a). Extensive use of this drug in the treatment of oxyuriasis has confirmed its safety (White and Standen, 1953a and b). A trial of single dose therapy was made in Africa by Goodwin and Standen (1954), with dosage ranging from 1 to 4 gm. for children and adults. In most instances, treatment was followed by a saline purge. Post-treatment stool specimens were obtained five to seven days after treatment from 66 of those treated. Of these, 48 were found to be free of *Ascaris* eggs, representing a cure rate of 73 per cent.

Piperazine citrate thus appeared to be the most promising ascarifuge for single dose therapy and was elected for use in this study. In addition to determining the effectiveness of piperazine in single dosage as a practical public health measure, it was anticipated that this study would throw light on the following problems: (1) the importance of post-treatment purgation; (2) the evaluation of a dosage schedule in different weight groups; (3) the relationship of the severity of infection (worm load) and efficacy of treatment.

METHODS

Several mining camps and rural areas in eastern Kentucky were studied. Cardboard cartons were labelled with the names of all persons living in these areas and were distributed to their homes; fifty to ninety per cent were subsequently collected. By utilizing the Beaver direct smear method (Mackie *et al.*, 1954), a 2 mg. sample of each stool was examined for a single count of *Ascaris* eggs. All persons found positive for *Ascaris* were treated in special clinics or in their homes on designated days. Each person was weighed and his drug dose calculated according to his weight group. The size of the group treated on individual occasions varied from a few to as many as 80 persons. Treatment was supervised to insure complete ingestion of the drug. The therapeutic agent used was Burroughs Wellcome "Antepar" Citrate, containing piperazine citrate in an amount equivalent to 100 mg. of piperazine hexahydrate per cc. In each study area, over 90 per cent of the persons with stools found positive for *Ascaris* were treated.

Two to four days after treatment each home was visited by a field worker and an inquiry made concerning any untoward side effects of therapy, as well as the number of worms passed by treated persons after receiving the drug. Twelve to nineteen days after treatment, a stool specimen was requested from each treated person for examination by the method described above. Eighty-eight per cent of the treated individuals submitted a post-treatment sample so that the effectiveness of the drug could be checked. Persons found to be still positive for *Ascaris* were treated a second time.

Preliminary studies were made on a group of approximately 60 persons to

TABLE 1

Dosage schedules employed

Weight in lbs.	Piperazine Citrate			Phenolphthalein (Laxative)
	Dosage 1	Dosage 2	Dosage 3	
15–20	10 cc.	10 cc.	10 cc.	30 mg.
21–30	20 cc.	15 cc.	15 cc.	60 mg.
31–50	25 cc.	20 cc.	15 cc.	120 mg.
51–100	30 cc.	25 cc.	20 cc.	180 mg.
101–150	40 cc.	30 cc.	25 cc.	240 mg.
151 and over	40 cc.	35 cc.	30 cc.	300 mg.

develop practical methods for this study and to determine a satisfactory dosage schedule. In regard to the latter, the dosage of piperazine used by Goodwin and Standen (1954) in Africa, and a treatment schedule utilized by Dr. H. W. Brown (unpublished data), were combined and modified in such a way that each dose was proportional to body surface area, as described for other drugs by Crawford *et al.* (1950). For practical purposes, dosage was adjusted beforehand according to weight groups, thus avoiding individual calculations at the time the drug was administered. This was deemed advisable to facilitate conduct of a mass treatment program. In the preliminary study, a preponderance of cures was noted in larger persons. Consequently, dosage in children weighing under 50 lbs. was increased, resulting in the first dosage schedule outlined in Table 1. Dosage schedules 2 and 3 (Table 1) were determined by successively decreasing piperazine dosage in any weight group where side effects were reported with the preceding dosage schedule.

In approximately half of the study areas, each person treated was given an appropriate amount of phenolphthalein, to be taken before breakfast on the morning after treatment. This laxative was reported to be uniformly effective in the majority of persons treated. Since it is known that piperazine temporarily paralyzes ascarides (Goodwin and Standen, 1954; Swartzwelder *et al.*, 1955a; Etteldorf and Crawford, 1950), it appeared that a laxative might increase the efficacy of piperazine by increasing the rate of evacuation of paralyzed ascarides from the bowel. The validity of this theory, at least with respect to the particular laxative used, is discussed below.

RESULTS

A geometric mean count of *Ascaris* eggs before treatment in the entire study was 6.0 eggs per mg. of feces. The range of pretreatment counts among the four groups in which therapy differed was 4.8 to 6.8 (Table 2). Results of the various dosage schedules were considered to be comparable within this range. The relative efficacy of the different modes of treatment was determined in four categories according to the reduction in egg-count among 282 persons from whom follow-up stools were procured. Post-treatment fecal specimens were examined and cure rates in the four groups were found to vary from 62.1 to 79.3 per cent

TABLE 2

Relative efficacy of three different therapeutic dosages of piperazine citrate and pretreatment geometric mean counts of eggs per mg. of feces

Weight in lbs.	Dosage 1			Dosage 2						Dosage 3		
	Without laxative			Without laxative			With laxative			With laxative		
	Total in group	No. cured	Per cent cured	Total in group	No. cured	Per cent cured	Total in group	No. cured	Per cent cured	Total in group	No. cured	Per cent cured
A. Per cent of individuals cured*												
15–20							2	1	50.0	2	0	
21–30	5	3	60.0	7	4	57.1	16	7	43.8	10	6	60.0
31–50	22	16	72.7	17	16	94.1	40	24	60.0	23	13	56.5
51–100	12	10	83.3	30	23	76.7	37	24	64.9	27	20	74.1
101–150	5	4	80.0	3	2	66.7	7	7	100.0	10	5	50.0
151+				1	1	100.0	1	1	100.0	5	5	100.0
Total	44	33	75.0	58	46	79.3	103	64	62.1	77	49	63.6

B. Pretreatment geometric mean egg counts			
Dosage 1	Dosage 2		Dosage 3
6.0	4.8	6.0	6.8

C. Per cent reduction in egg counts† among individuals not cured			
50.0	60.0	62.5	63.8

D. Average interval in days between therapy and post treatment stool collection			
15.4	18.9	17.0	12.3

* Criterion used for cure is reduction of counts of eggs to zero as determined by a single post treatment fecal smear of 2 mg. utilizing the Beaver direct smear method.

† Determined by the difference between pretreatment and post-treatment geometric mean counts of eggs.

(Table 2, Figure 1). Individuals with counts not reduced to zero showed reductions ranging from 50 per cent with Dosage 1 to 63.8 per cent with Dosage 3. Re-treatment with two doses, one day apart, was effective in the great majority of instances where single doses did not cure.

Dosage 2 without a laxative produced complete reduction of egg counts to zero in 79.3 per cent of the treated individuals. A reduction of the original count to one-fourth or less was attained in 86 per cent of the group and in 91 per cent the counts were cut to half or less. The other schedules were not as successful as Dosage 2 minus laxative.

Doses of piperazine in the same weight groups differed as much as 60 per cent among Dosages 1, 2, and 3. However, reduction in counts of eggs with Dosage 1, the heaviest schedule, were not significantly different from reductions by means of Dosage 2 without a laxative. The same lack of significance was evident in comparing Dosage 2 with a laxative, and Dosage 3 also with a laxative (Fig. 1).

The cure rate was 17.1 per cent lower when a laxative was used in conjunction with Dosage 2 than when it was omitted. This proved to be a significant difference ($p = 0.02$).

Reductions in egg counts were comparatively greater in individuals with heavier worm burdens, but no critical count was recognized as pinpointing effectiveness of treatment relative to intensity of infection. However, when compared in terms of reductions of counts to zero, 70 per cent of persons with pretreatment counts of less than 35 per mg. of feces were cured, whereas 48 per cent with pretreatment counts above 35 were cleared ($p = 0.02$). The number of individuals with counts at higher levels was inadequate for such comparison. Table 2 indicates that rates of cure were somewhat lower in small children and tended to approach 100 per cent in adults. It was observed also that heavier infections of *Ascaris* generally occurred among young children. A majority of the treated individuals reported the numbers of roundworms that were passed, varying from a very few to over 100 specimens. These observations were interesting, but they were not considered satisfactory for analysis.

Side effects of piperazine therapy occurred at random without significant body-weight grouping (Table 3). All were minor with the exception of vomiting in less than 18 hours after administration of this drug, and included dizziness, weakness, headache, and mild abdominal pain within 24 hours after treatment. Rarely were side effects of any concern and all were of brief duration. By slightly decreasing the dosage, as mentioned previously, side effects were markedly diminished without decline in the efficacy of the piperazine.

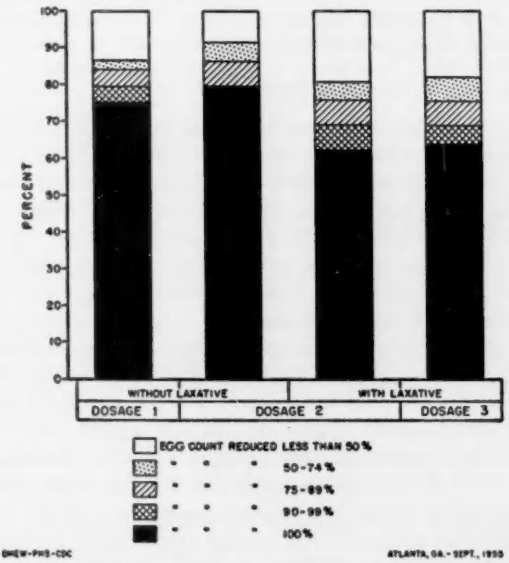


FIG. 1. Relative efficacy of three different therapeutic dosages of piperazine citrate as determined by ascaris egg counts.

TABLE 3

Side effects of therapy

Weight in lbs.	Dosage 1			Dosage 2					Dosage 3		
	Without laxative			Without laxative			With laxative			With laxative	
	No. individuals questioned	No. reporting		No. individuals questioned	No. reporting		No. individuals questioned	No. reporting		No. individuals questioned	No. reporting	
		Vomit*	Minor side effects†		Vomit	Minor side effects		Vomit	Minor side effects		Vomit	Minor side effects
15–20							2		1	2		
21–30	4		1	7			16			6		
31–50	20	1	2	16	1	1	39	1	2	27		
51–100	12	1	2	28	1	1	36	2	4	27	1	
101–150	5		3	3	1		6		2	10		1
151+				1			1			5		
Total....	41	2	8	55	3	2	100	3	9	77	1	1
Per cent reporting side effects		4.9	19.5		5.5	3.6		3.0	9.0		1.3	1.3

* Vomiting occurring 3–18 hours after treatment with piperazine citrate.

† Minor side effects include dizziness, weakness, headache, mild abdominal pain and mild abdominal cramps occurring within 24 hours after treatment.

No beneficial effects from piperazine therapy were noted against other helminths in 147 individuals who, in addition to an *Ascaris* infection, were parasitized by one or more of the following: *Trichuris trichiura*, *Strongyloides stercoralis*, *Hymenolepis nana*, and hookworm.

DISCUSSION

The geometric mean egg count was used to evaluate the intensity of infection for each study group as a whole since this figure minimized the distorting effects of a few extreme individual counts. Wide variations in mean intensities between the groups would have complicated comparison of different dosages as to efficacy.

Omission of a laxative with Dosage 2 enhanced the per cent of persons cured, and this may prove to be true also with Dosage 3 which is somewhat lighter. Since reports of side effects with Dosage 3 were virtually negligible, further studies will be made with this lower schedule.

It was observed, in connection with preliminary studies previously mentioned but not detailed, that a dearth of affirmative reports concerning side effects was noted unless inquiries were made not longer than four days after treatment. This tended to minimize the significance of such effects as were reported.

A cure rate of 79 per cent, the best result in this study, compares favorably with the findings (73 per cent) of Goodwin and Standen (1954) who also used a single dose of piperazine citrate. In a recent paper by Swartzwelder, *et al.* (1955b), a single dose treatment with piperazine citrate in syrup was reported completely effective in 16 of 17 children treated for ascariasis, 11 of whom were hospitalized and 6 treated in clinics. Brown (1954) obtained a 90 per cent cure rate among

51 children with multiple doses of the same drug over a 2 to 5-day period. Approximately the same result was reached by Swartzwelder *et al.* (1955) with 25 patients given multiple doses. The moderate additional success reportly attendant upon a series of doses is outweighed in mass treatment by the convenience and reliability of administering the single dosage under observation to ascertain that the drug is actually taken.

SUMMARY AND CONCLUSION

Single doses of piperazine citrate were administered during mass treatment of human ascariasis in five study areas in the mountains of eastern Kentucky. A total of 282 persons was treated in four groups with three different dosage schedules, two groups receiving a laxative after treatment. In the more successful laxative group, 64 per cent of the individuals had egg counts reduced to zero as determined by observations before and after treatment. However, in another group omitting the laxative, a cure rate of 79 per cent was obtained. Reports of side effects were few and judged to be of little concern. Piperazine citrate in single dosage is considered an effective public health measure for use in control of roundworm infections.

REFERENCES

Belding, D. L., 1952. *Clinical Parasitology*. Appleton-Century-Crofts, New York, pp. 1139.

Brown, H. W., 1954. The treatment of *Ascaris lumbricoides* infections with piperazine, *J. Pediat.* **45**: 419–424.

Brown, H. W., and Sterman, M. M., 1954. Treatment of *Ascaris lumbricoides* infections with piperazone citrate, *Am. J. Trop. Med. & Hyg.* **3**: 750–754.

Crawford, J. D., Terry, M. E., and Rourke, G. M., 1950. Simplification of drug dosage calculation by application of the surface area principle, *J. Pediat.* **5**: 783–789.

Etteldorf, J. N., and Crawford, L. V., 1950. Treatment of ascariasis in children, *J. A. M. A.* **143**: 797–799.

Gabbard, M. B., Kotcher, E., and Pulliam, E. D., 1954. Evaluation of hetrazan as an anthelmintic in children, *Pub. Health Rep.* **69**: 1087–1089.

Goodwin, L. G., and Standen, O. D., 1954. Treatment of roundworm with piperazine citrate ("Antepar"), *Brit. M. J.* **2**: 1332–1333.

Hoekenga, M. T., 1952. Treatment of ascariasis in children with hetrazan syrup, *Am. J. Trop. Med. & Hyg.* **1**: 688–692.

——— 1954. Experiments in the therapy of human ascariasis, *Am. J. Trop. Med. & Hyg.* **3**: 755–761.

Mackie, T. T., Hunter, G. W. III, and Worth, C. B., 1954. *A Manual of Tropical Medicine*. W. B. Saunders, Philadelphia, pp. 907.

Oliver-González, J., Santiago-Stevenson, D., and Hewitt, R. I., 1949. Treatment of six cases of ascariasis in man with diethyl-carbamyl-4-methyl piperazine hydrochloride, *South Med. J.* **42**: 65–66.

Swartzwelder, C., Miller, J. H., and Sappenfield, R. W., 1955a. The treatment of cases of ascariasis with piperazine citrate, *Am. J. Trop. Med. & Hyg.* **21**: 326–331.

———, 1955b. Treatment of ascariasis in children with a single dose of piperazine citrate, *J. Pediat.* **16**: 115–117.

White, R. H. R., and Standen, O. D., 1953a. Piperazine in the treatment of threadworms in children, *Brit. M. J.* **2**: 755–757.

———, 1953b. Piperazine in the treatment of threadworms, *Brit. M. J.* **2**: 1272–1273.

AN EVALUATION OF THE STANDARDIZED DIRECT SMEAR FOR EGG COUNTING IN PARASITOLOGICAL WORK[1]

JOSÉ F. MALDONADO

Department of Microbiology, School of Medicine—School of Tropical Medicine, San Juan, Puerto Rico

Egg counting is a necessary complement to fecal examinations in parasitological work in order to determine the approximate severity and importance of helminth infection. Beaver's standardized direct smear (1949, 1950), because of its simplicity, may be of great aid in large scale surveys but not much has been published respecting its use. Aside from Beaver's original contributions, Melvin and co-workers (1955) found the technique efficient in estimating the magnitude of hookworm infection when compared with the Stoll dilution egg count (1926a, 1926b).

The present investigation was carried out to compare the standardized smear with the Stoll technique as applied on a wide scale to the various human intestinal helminths common to Puerto Rico.

MATERIALS AND METHODS

The investigation was based on 1,000 fecal samples obtained from all age groups over a period of six months from various localities. The number of infections found in these samples was as follows: *Ascaris lumbricoides*, 411; *Trichuris trichiura*, 951; *Necator americanus*[2], 449; *Schistosoma mansoni*, 317. The majority of the stools were of a formed character. A standardized smear with a density equivalent to 5 mgm. and a Stoll and Hauscheer count based on a single 0.075 ml. drop of stool suspension (which corresponds to 5 mgm. of formed stool) were made from each sample. The specimens were refrigerated after collection and were from two to five days old at the time the examinations were made. Each sample was well comminuted with a wooden spatula before being processed. The number of eggs for each species of worm and the character of the feces were recorded. The latter was used to convert the readings with the Stoll and Hauscheer count to a formed stool basis with a factor of 1 for formed, 1.5 for formed-mushy, 2 for mushy and 4 for diarrheic samples. For uniformity the character of the feces was recorded by the same individual.

Both Beaver's (1949, 1950) and Stoll and Hauscheer's procedures (1926a, 1926b) were followed without modification. The photometer employed for the first technique was a Weston Master II Universal Model 753, calibrated and checked intermittently with $BaSO_4$ suspensions supplied by Beaver or prepared by the author.

Coefficients of correlation were calculated according to the standard statistical

[1] The present work has been carried out with the partial support of the Department of the Army Contract No. DA-49-007-MD-24.

[2] *A. duodenale* was disregarded since this species occurs in negligible numbers in Puerto Rico (Oliver-González *et al.*, 1949).

procedure, with X representing the counts by the standardized direct smear and Y by the dilution technique. In order to present the results in a more comparative manner, however, the data were analyzed using the χ^2 relationships by the graphic method. For this purpose it was necessary to transform the number of eggs into light, medium and heavy parasitization. For *A. lumbricoides* a light infection (L) represented up to 5,000 eggs per ml. of feces; medium (M), 5,000 to 20,000 eggs per ml. of sample; and heavy (H), 20,000 eggs and over per ml. of sample. These rates are based on approximately 1,000 eggs per ml. of stools per worm (Brown and Cort, 1927; Augustine *et al.*, 1928). For *T. trichiura* L represents 10,000 eggs or less per ml. of feces, M. represents 10,000 to 20,000 per ml. of sample and H represents 20,000 eggs or more per ml. of sample. These figures are based on a rate of approximately 300 eggs per ml. per worm (Burrows, 1950). For *N. americanus*, L represents 5,000 eggs or less per ml., M represents 5,000 to 20,000 eggs per ml. and H 20,000 or more eggs per ml., calculated on 50 eggs per ml. of feces per adult worm (Stoll and Hauscheer, 1926a, 1926b). For *S. mansoni*, because of the nature of the parasitosis and the difficulty in correlating egg number with degree of parasitization, egg counts were arranged in units of 1,000 eggs per ml. of feces for comparison.

RESULTS

Seventy-nine per cent of the fecal samples were of the formed type. If to these are added those with a mushy portion (with a 1.5 conversion factor) the number of stools in which the Stoll and Hauscheer drop of suspension represented 5 mgm. increases to 83 per cent.

Table 1 analyzes the results in general and, to take into account consistency of the feces, utilizes coefficients of correlation. The consistency of the samples had no influence on the coefficients for *A. lumbricoides*, *N. americanus* and

TABLE 1

Coefficients of Correlation for the Stoll and Hauscheer Technique and Beaver's Standardized Fecal Smear Method

	Ascaris	Trichuris	Hookworm	Schistosoma
Total egg counts	411	951	449	317
Coefficient of correlation	0.87	0.71	0.52	0.57
Formed feces				
No. of counts	336	699	346	234
Coef. of Correl.	0.87	0.75	0.52	0.57
Formed-mushy feces				
No. of counts	17	40	33	14
Coef. of Correl.	0.93	0.78	0.73	0.56
Mushy feces				
No. of counts	50	200	75	65
Coef. of Correl.	0.84	0.37	0.66	0.59
Liquid feces				
No. of counts	8	12	4	4
Coef. of Correl.	0.99	0.97	0.94	0.97

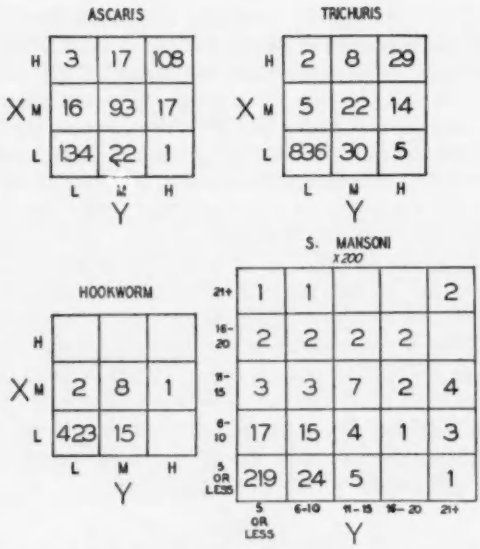


FIG. 1. χ^2 relationships of standardized smear and Stoll egg counting methods
X = Smear Y = Stoll

S. mansoni, but in the case of *T. trichiura* an unexplainably low figure was associated with mushy feces.

Figure 1 represents the findings as analyzed by the χ^2 graphic method. For *A. lumbricoides* both counting methods agreed on 335 of 411 (81 per cent) of the samples with a highly significant coefficient of correlation of 0.87. The average number of eggs per ml. of feces counted by the standardized smear was 20,000, and was 22,400 by the dilution egg counting technique. On the basis of 1,000 eggs per ml. of feces per worm (Brown and Cort, 1927; Augustine *et al.*, 1928) the calculated worm burden per person was 20 and 22, respectively.

With *T. trichiura* both techniques showed a correlation of 93 per cent by the χ^2 method of analysis with only a negligible number (0.7 per cent) of widely divergent counts. The coefficient of correlation was .71 (significant, but not highly so). The average number of eggs per ml. of feces for this parasite counted by the standardized direct smear was 5,400, against 4,400 for the dilution counting method. The corresponding number of worms represented by these two figures are 18 and 15, respectively.

With *N. americanus* the techniques agreed in 431 of the 449 samples, or 96 per cent (Fig. 1), and the coefficient of correlation was 0.52, considerably lower than for the previous worms. The average number of eggs counted by Beaver's technique was 1,600 per ml. of feces, against 800 by the dilution egg count. These figures represent a calculated worm burden of 32 *N. americanus* per parasitized individual by the first and 16 worms by the second technique.

For *S. mansoni* both counting methods coincided in 245 of the 347 counts (80 per cent). The divergences encountered were slight for the most part. The coefficient of correlation was 0.57, as low as for *N. americanus*. The average number of eggs per ml. of feces for the standardized smear was 860 and 800 for the dilution egg count.

DISCUSSION

Certain considerations must be taken into account in the evaluation of the data. It is the general opinion that the 2 mgm. preparation is the most convenient one to employ in executing the standardized direct smear, since considerable time is saved in the counting of eggs without sacrificing accuracy. Under the circumstances of the investigation, however, with over 80 per cent of the fecal samples of a formed type, the best smear to use in a comparison with the Stoll method was an equivalent one, namely, a 5 mgm. preparation. To a certain extent a smear standardized to 5 mgm. is thick and the technician has to take care in making the counts, since *A. lumbricoides* and *N. americanus* eggs are easily overlooked or confused with artifacts. Our experience has been, however, that the thickness of the preparation did not interfere with the results of the survey, the average egg counts by both methods being essentially similar. It may appear that with *N. americanus* there is a wide difference in this respect, with an average of 1,600 eggs per ml. of feces for Beaver's method against 800 for Stoll's, but divergences at such low levels of infection should hardly be considered significant.

In survey work the main goal of egg counting is to determine the rates of light, medium and heavy parasitization. The best method of evaluating the two techniques was, therefore, by χ^2 graphs since the number of samples in which both coincided and differed was made evident. The data for *A. lumbricoides* were best for complete analysis, since all degrees of parasitization were well represented. This has resulted in highly significant statistical values for this parasite. The low values obtained in the coefficients of correlation with *T. trichiura*, *N. americanus* and *S. mansoni* were due to the predominance of very small numbers of eggs and may not be considered highly significant by some statisticians.

The difference in the average number of worms calculated to be present by both methods was negligible. The selection of the method therefore, appears to depend on which one is simpler and requires less laboratory facilities. In our experience the Beaver technique is less time consuming, is easily done, and is not affected by the consistency of the stool. As a routine laboratory method it is believed that the standardized smear provides the technician with a qualitative as well as a quantitative measure of worm infections.

SUMMARY AND CONCLUSIONS

The standardized direct smear and the Stoll dilution egg counting methods were compared in 1,000 fecal samples. Of these 411 had shown *A. lumbricoides*, 951 *T. trichiura*, 449 *N. americanus* and 310 *S. mansoni* eggs.

The data obtained with the two methods were analyzed by the χ^2 test and coefficient of correlation. In general the techniques compared favorably. The *A. lumbricoides* was represented by all degrees of intensity of infection and gave highly significant results. A majority of the infections with the other three helminths were light. It is believed that this led to the less significant results obtained with these species. It is concluded that the standardized smear is the technique of choice.

REFERENCES

Augustine, D. L., Nazmi, M., Helmy, M., and McGavran, E. G., 1928. The ova-parasite ratio for *A. duodenale* and *A. lumbricoides*, *Jour. Parasitol.* **15:** 45–51.

Beaver, Paul C., 1949. Quantitative hookworm diagnosis by direct smear, *Jour. Parasitol.* **35:** 125–135.

Beaver, Paul C., 1950. The standardization of fecal smears for estimating egg production and worm burden, *Jour. Parasitol.* **36:** 451–456.

Brown, H. W., and Cort, W. W., 1928. The egg production of *A. lumbricoides*, *Jour. Parasitol.* **14:** 88–90.

Burrows, R. B., 1950. On the estimation of *Trichuris* worm burden in patients, *Jour. Parasitol.* **26:** 227–231.

Melvin, D. M., Sadun, E. H., and Heimlich, R., 1955. Evaluation of the reliability of the dilution egg count and the direct smear egg count in the quantitative determinations of hookworm infections, *Jour. Parasitol.* **41:** (6), Sec. 2, Suppl. 42.

Oliver-González, J., Dobal, J. M., and Thillet, C. J., 1949. *N. americanus* and *A. duodenale* infections in Puerto Rico, *Jour. Parasitol.* **35:** (6), Sec. 2, Suppl.

Stoll, N. R., and Hauscheer, W. C., 1926a. Accuracy in the dilution egg counting method, *Am. Jour. Hyg.* **6:** March Suppl., 80–193.

Stoll, N. R., and Hauscheer, W. C., 1926b. Concerning two options in dilution egg counting: small drop displacement, *Am. Jour. Hyg.* **6:** March Suppl. 136–145.

MURINE TYPHUS FEVER AND RAT ECTOPARASITES IN PUERTO RICO

IRVING FOX

Department of Microbiology, School of Medicine—School of Tropical Medicine, San Juan, Puerto Rico

To control murine typhus fever in southern United States large scale projects involving in particular DDT dusting to kill rat fleas were instituted in late 1945. During the years which followed the number of cases reported dropped steadily and many articles appeared in which the reduced rate is attributed to control. In Public Health Reports for 1952 under the title of "Trends", one reads, "The incidence of endemic typhus fever, which has occurred principally in southeastern United States, has decreased. The lower incidence shown for 1951 undoubtedly has resulted from control measures applied on a fairly extensive scale in a number of States". Pratt and Good (1954) state, "The success of this control program (which included rat poisoning, rat trapping, and ratproofing, but consisted primarily of DDT dusting) may be measured by the remarkable decrease in the number of cases of typhus in the 8 years since 1944." However, it has been pointed out that murine typhus like other infectious diseases has natural epidemic peaks and recessions, so that control measures may not necessarily have been entirely responsible for the decrease in incidence (Rickard and Wiley, 1948).

In view of the situation in Puerto Rico, statements which ascribe a reduced typhus rate to control are puzzling, for here control measures in no way even approximated those practiced in the southern states. DDT dusting operations were not undertaken and control efforts involved only attempts at ratproofing and rat exterminating on a minor scale in certain localities under a very small budget. Trapping of live animals during this control period, personal observations of damage done by rats and continuous complaints of the citizens demonstrated that these measures were not effective in making an appreciable reduction in the rat population which has always been great. Nevertheless, the number of cases reported, most of which came from the city of San Juan, dropped from 189 in 1944 to 5 in 1952 representing a 97 per cent reduction, or one greater than that which occurred in the continental United States. In the city of San Juan the reported rate decreased 100 per cent from 1944 to 1954 (Fig. 1).

At the outset, it was clear that any explanation of the typhus situation in Puerto Rico would depend upon new data acquired from rat ectoparasite and serological surveys which could be compared with information obtained when the disease was a serious problem. In 1945, 54 per cent of the rats of San Juan were positive for murine typhus by the complement-fixation test (Pomales Lebrón *et al.*, 1946) and an ectoparasite survey made in 1946 indicated that more than one-half of the rats were infested with *Xenopsylla cheopis* (Fox, 1951). If another study demonstrated that rats have as many fleas as formerly and are infected with typhus to the same degree, then accepted epidemiological concepts are

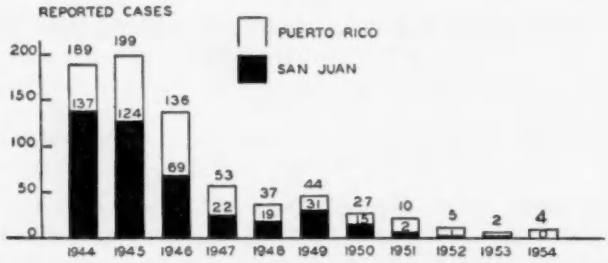


FIG 1

FIG 2

	R.R.	R.N.	INF.%	MEAN
LA PERLA	87	23	29	0.61
SAN JUAN	80	9	11	0.69
P TIERRA	68	33	37	0.99
S J ISLET	235	65	26	0.76
SANTURCE	15	285	5	0.08

FIG. 3

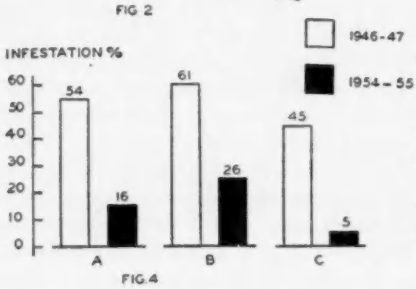


FIG. 4

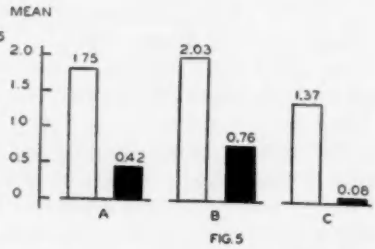
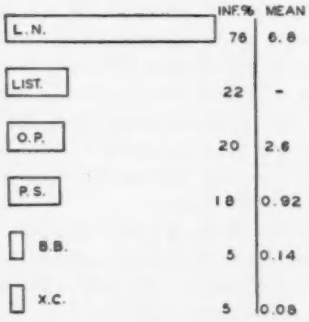
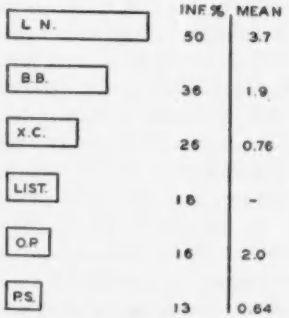


FIG. 5



SANTURCE

FIG. 6



SAN JUAN ISLET

FIG. 7

FIGS. 1–7

wrong or there are many hidden human cases. If, on the other hand, a new study showed that flea infestation and typhus infection had greatly decreased among the rats, the present low human morbidity would be explained; but at the same time there would be posed the problem as to why this had taken place in the absence of adequate rat and flea control operations. Accordingly, an ectoparasite survey was undertaken and 600 rats were trapped alive in the city of San Juan from April 15, 1954, through May 4, 1955, using wooden box type traps set in places heavily infested with rats and the data obtained were compared with those from 775 rats trapped during the same period in 1946–1947 (Fox, 1951).

A narrow, well bridged channel separates the older portion of San Juan called San Juan Islet from the near mainland called Santurce (Fig. 2). San Juan Islet includes the Old City which is largely devoted to business but also has tenement type apartment houses, a heavily populated district of poor small habitations called La Perla, and an intermediate not fully constructed district known as Puerta de Tierra. Santurce is largely residential, its poorer sections although crowded consist of small wooden houses with unpaved ground around them. The rat populations of San Juan Islet and Santurce are remarkably different, for in the former *Rattus rattus*, with its various color phases predominates, while in the latter *R. norvegicus* is the predominant species. The number of rats captured in the 1954–55 survey by calendar month and species is shown in Table 1; and Table 2 gives these data for the 775 rats captured during the same period in 1946–47. As regards Santurce the rats in general came from the same localities in the two surveys, but on San Juan Islet so great were the changes due to business expan-

---

EXPLANATION OF PLATE

FIG. 1. Reported cases of murine typhus fever in Puerto Rico according to the Bureau of Communicable Disease Control, Department of Health, Commonwealth of Puerto Rico; blackened portions of each bar represent the number of cases reported from the City of San Juan. FIG. 2. Map of the City of San Juan indicating the districts and with stippling to show the general areas from which were collected 600 rats from April 15, 1954, through May 4, 1955. FIG. 3. Table giving the number of rats taken from each of the districts shown in Fig. 2, the species of rat (*Rattus rattus* or *R. norvegicus*), the percentage of rats infested with *Xenopsylla cheopis* (infestation per cent) and the average number of specimens of *X. cheopis* per rat (mean). FIG. 4. The abundance of *X. cheopis* as shown by infestation per cent in 1946–47 compared with the abundance in 1954–55; open bar 1946–47, filled bar, 1954–55. A.—San Juan Islet plus Santurce based on 775 rats collected from April 15, 1946, through May 4, 1947, and 600 rats collected during the same period in 1954–55. B.—San Juan Islet, based on 424 rats collected in 1946–47 and 300 rats in 1954–55. C.—Santurce, based on 351 rats collected in 1946–47 and 300 rats in 1954–55. FIG. 5. The abundance of *X. cheopis* as shown by the mean in 1946–47 compared with the abundance in 1954–55. A.—San Juan Islet plus Santurce. B.—San Juan Islet. C.—Santurce. FIG. 6. The relative abundance of *Laelaps nuttalli*, *Listrophoroides* species, *Ornithodorus puertoricensis*, *Polyplax spinulosa*, *Bdellonyssus bacoti*, and *X. cheopis* as shown by infestation per cent (the mean being given in the column to the right) based on 300 rats from Santurce collected in 1954–55. FIG. 7. The relative abundance of the same species as in FIG. 6 as shown by the infestation per cent (the mean being given in the column to the right) based on 300 rats taken in 1954–55 from San Juan Islet (specimens of *Listrophoroides* species were often too numerous to count accurately so that the mean was not determined).

TABLE 1

Number of rats captured each month in Santurce and San Juan Islet, Puerto Rico from April 15, 1954 through May 4, 1955

Month	Santurce		San Juan Islet		Total
	R. norvegicus	*R. rattus*	*R. norvegicus*	*R. rattus*	
April	13	0	0	14	27
May	40	0	3	11	54
June	96	4	1	2	103
July	4	1	15	31	51
August	20	0	13	17	50
September	29	2	4	16	51
October	32	4	2	17	55
November	6	1	7	27	41
December	16	2	1	27	46
January	14	1	2	16	33
February	5	0	12	11	28
March	10	0	3	23	36
April	0	0	2	23	25
Total	285	15	65	235	600

TABLE 2

Number of rats captured each month in Santurce and San Juan Islet, Puerto Rico from April 15, 1946 through May 4, 1947

Month	Santurce		San Juan Islet		Total
	R. norvegicus	*R. rattus*	*R. norvegicus*	*R. rattus*	
April	22	0	1	8	31
May	25	4	3	51	83
June	34	0	3	43	80
July	25	0	2	64	91
August	13	2	9	49	73
September	18	6	11	35	70
October	33	6	8	39	86
November	10	6	24	40	80
December	28	9	0	8	45
January	38	4	0	9	51
February	12	2	1	13	28
March	22	3	0	1	26
April	21	8	2	0	31
Total	301	50	64	360	775

sion during the eight years interval between the two surveys that it was quite impossible to collect the rats from the same places. In the 1946–47 survey unbaited steel traps were used in Old San Juan restaurants while in 1954–55 baited wooden box traps were used in tenement houses for the most part; in 1946–47 no trapping was done in La Perla while in 1954–55 many of the rats came from

this district; and finally the Puerta de Tierra localities differed in 1954–55 from those of the 1946–47 survey. To compensate for differences in sampling, a policy of bias in favor of places where rats had fleas was followed, so that errors have very likely been on the side of showing a greater flea infestation than actually exists. The map in Figure 2 shows the geographic features of the city of San Juan with stippling to indicate the localities in general from which the rats came, and the table in Figure 3 gives the numbers of rats caught in each district, the species of rats, the percentage infested with *Xenopsylla cheopis*, and the average number of specimens per rat, or the mean. The infestation per cent was highly variable on San Juan Islet and much depended on where the rats were caught, flea distribution being spotty rather than general. The high infestation per cent shown for the Puerta de Tierra district is due mainly to one large property where rats were well provided with fleas. Despite every effort to secure rats with fleas in Santurce the infestation per cent was very low. Figures 4 and 5 compare the data from the surveys made in 1946–47 and 1954–55 as regards the per cent of rats infested with *X. cheopis* and the mean for San Juan Islet plus Santurce (Figs. 4,A, and 5,A), San Juan Islet (Figs. 4,B and 5,B), and Santurce (Figs. 4,C and 5,C). The greatest reduction in flea population occurred in Santurce and this is consistent with the downward trend shown by earlier surveys when the *cheopis* infestation per cents were 51, 29 and 14 for the calendar years 1946, 1947 and 1948 respectively (Fox, 1951). The *cheopis* infestation per cent for San Juan Islet is still rather high although much lower than in 1946–47 (Fig. 4,B) and it is interesting to note that if in 1946 San Juan Islet had been selected as a check area and Santurce as a DDT dusting area we would now say that DDT dusting is very effective and might even claim that the effects of DDT dusting lasts for several years.

Five species of ectoparasites in addition to *X. cheopis* were common on the rats of the 1954–55 survey, *Laelaps nuttalli*, an unidentified species of *Listrophoroides*, *Bdellonyssus bacoti*, *Polyplax spinulosa* and *Ornithodorus puertoricensis* (Fox, 1947) and their relative abundance on the basis of infestation per cent is shown in Figures 6 and 7. When the data from Santurce are compared with the situation in 1946 we find that *X. cheopis* dropped from second place to last place and each of the other ectoparasites except *O. puertoricensis* increased in abundance; however infestation with this tick (20 per cent) was greater than it was in 1948 (16 per cent). Not shown in the graph is *Echidnophaga gallinacea* which in the 1946 survey infested 20 per cent of the rats but only 2 per cent in 1948; in 1954–55 the infestation per cent with this flea was 1.3 per cent and the mean 0.017. As regards San Juan Islet, *X. cheopis*, when compared with the situation in 1946, dropped from first place to third place and the other ectoparasites, except *O. puertoricensis*, which decreased from 21 per cent to 16 per cent, showed great increases. *E. gallinacea* remained about the same as it was in 1946, with an infestation per cent of 1.3 and a mean of 0.077. These data are significant in that they indicate that no other ectoparasite except *X. cheopis* plays an important part in the transmission of murine typhus to man.

The results of this ectoparasite survey, when compared with previous ones,

demonstrate that the rat flea population decreased to a degree commensurate with the low human murine typhus rate and this confirms accepted opinion concerning the epidemiology of the disease. It is possible that improvements in construction during the last decade although not reducing the rat population did affect the habitats in such a way as to retard flea development, and consideration must also be given to insecticidal operations against mosquitoes such as larviciding for *Culex*, use of a fogging apparatus and partial DDT residual spraying of houses against *Aedes aegypti*. These aspects are discounted, however, because it is unlikely that larviciding would have any effect on rat fleas, while fogging (which began *circa* 1952) and residual spraying (*circa* 1954) were instituted after the incidence of the disease had already reached a very low point and were not accomplished in a manner likely to influence rat fleas. Moreover it is reasonable to suppose that if mosquito control and improved construction had reduced the fleas they would also have had a deterrent effect on other common rat ectoparasites, which was not the case. Climatic factors, particularly the average January temperature, according to Mohr *et al.* (1953), influence the distribution of murine typhus in the United States where, the disease is thought to be widespread among rats on isolated premises which have not been subject to control in areas with an average January temperature above 47°F. and a high humidity, such conditions being favorable for the development of the flea vector. In San Juan the difference between the average temperatures of the warmest and coolest months is only about 5.6° and the lowest temperature ever recorded was 62°; in 1946 the average January temperature was 75.2° and the January relative humidity varied between 77 and 89 per cent, while in 1954 the average January temperature was 75.0° and the January relative humidity varied from 66 to 83 per cent (U. S. Dept. of Commerce, Weather Bureau, 1954). In the years between 1946 and 1954 there was little significant variation from these figures and the San Juan climate should have been very favorable for the persistence of the disease.

Human serology. Sera from 383 human subjects (blood bank donors for the most part) collected from April 19 to July 19, 1955, were submitted to the Sub-Department of Bacteriology, School of Medicine, University of Puerto Rico, where the complement-fixation test by the Kolmer method using Lederle endemic typhus antigen was made by Mrs. Maria Medina de Cermeño under the direction of Dr. Rafael Mariñelarena. Results were obtained on a total of 368 (8 were anticomplementary and 7 were lost) of which 14 or 3.8 per cent were positive at titers of 1:4 or above as follows: 1:4, 4 positive; 1:8, 2 positive; 1:16, 6 positive; 1:32, 1 positive; 1:64, 1 positive. Although the percentage positive is similar to that obtained in 1945 by Pomales Lebrón (1948) who found 3.5 per cent positive of 1,000 donors at dilutions of 1:4 or 1:8, it does not indicate that typhus is as prevalent today as formerly for the following reasons: (1) The blood specimens came from the metropolitan area of the City of San Juan, where typhus was formerly much more common than in Puerto Rico as a whole, as in Pomales Lebrón's series. (2) It is possible that a positive reaction may be given as many as ten years after recovery. (3) The low titers indicate that it is unlikely that cases of recently acquired infection are involved. These low titers are remarkable when compared with the results reported by Pomales Lebrón (1948),

who shows that the sera of 43 out of 53 endemic typhus fever cases gave titers of 1:32 or above, of whom 9 gave titers of 1:1,024 or above, when tested by Dr. Ida Bengston and Col. Harry Plotz using endemic typhus antigen.

Rat serology. Bloods from 578 rats collected from April 28, 1954, to July 26, 1955, were subjected to the complement-fixation test, but, unfortunately, results could not be obtained on a large number of these because of anticomplementary reactions. Of 279 only 9 were positive at 1:8 (1:8, 8 positive, 1:32, one positive) or only 3.2 per cent; and 89 were anticomplementary in dilutions of 1:4 and 1:8 and negative at 1:16. Pomales Lebrón *et al.* (1946) found that of 413 rats collected from December 7, 1944, to June 22, 1945, 54 per cent were positive to the complement-fixation reaction using Lederle epidemic typhus antigen, hence it is apparent that there has been a great reduction in infection among rats. Through the courtesy of Dr. Joseph H. Schubert, Serology Research Laboratory, U. S. Public Health Service, Chamblee, Ga., results were available on 147 rat sera collected in the City of San Juan from November 25, 1947, to June 18, 1948, and of these 7 or 4.8 per cent were positive (1:8, 2 positive; 1:16, one positive; 1:32, one positive; 1:64, one positive; 1:128, 2 positive) with the complement-fixation tests starting at dilutions of 1:8. This indicates that the decrease of infection in rats occurred even before 1948.

SUMMARY AND CONCLUSIONS

The remarkable reduction in murine typhus fever in southern United States is generally attributed to large scale control measures, particularly DDT dusting to kill rat fleas. Notwithstanding that such control operations were not in effect in Puerto Rico, the reported rate dropped steadily and there was a 100 per cent decrease in the City of San Juan (137 reported cases in 1944 and none in 1954). In an attempt to explain the decline of this disease a rat ectoparasite study together with human and rat serological surveys were undertaken. Ectoparasite data from 600 rats collected in the City of San Juan from April 15, 1954, through May 4, 1955, when compared with data from 775 rats trapped during the same period in 1946–47 demonstrated that the flea vector, *Xenopsylla cheopis*, had decreased in population sufficiently to account for the lack of human cases; for only 16 per cent of the rats in 1954–55 were infested with an average of 0.42 specimens per rat as compared with 54 per cent infested and 1.75 specimens per rat in 1946–47. Decrease in flea population was greater in the residential section known as Santurce where only 5 per cent of the rats were infested than in the largely business district, San Juan Islet, where 26 per cent of the rats were still infested. As regards Santurce, data from earlier surveys indicated that there has been a consistent downward trend in rat flea population during the last eight years. Complement-fixation tests on 279 rats resulted in only 3.2 per cent positive at dilutions of 1:8, indicating that infection among rats is negligible. Of 368 human subjects, 3.8 per cent were positive at dilutions of 1:4 or above using the complement-fixation test; since a positive reaction may be given years after recovery and because of very low titers as well as other reasons it is believed that the reported rate is essentially correct and there are few if any unreported cases.

On the basis of this evidence from Puerto Rico it is concluded that a reduced

typhus rate is not proof of the efficacy of control operations. Further, it is possible that control was instituted in southern United States after the epidemic had already receded, and that without the large scale control projects there the disease might have followed the same course it has in Puerto Rico. No explanation has yet been found for the decrease in the reproductive potential of rat fleas during the last decade in San Juan, P.R., since rats are abundant and the climate has been favorable for flea development, at least as regards temperature and humidity. Improvements in construction, food storing, general sanitation and mosquito control may have had an incidental effect on rat fleas, but do not account for the great reduction in population which has occurred.

ACKNOWLEDGMENTS

Thanks are due Dr. E. Harold Hinman for his encouragement and support of this study; Dr. R. A. Timothée Andraca for permission to use murine typhus morbidity data from the Department of Health, Commonwealth of Puerto Rico; Mrs. Maria Medina de Cermeño and Dr. R. Mariñelarena for running complement-fixation tests on rat and human sera; J. del Toro and J. Oliver-Ruiz, medical students who assisted the writer during the summers of 1954 and 1955; Dr. J. H. Schubert for permission to use U. S. Public Health Service serological data; M. Pérez Torres for information on mosquito control activities in San Juan; and Dr. A. Pomales-Lebrón for helpful advice.

REFERENCES

Fox, I., 1947. *Ornithodorus puertoricensis*, a new tick from rats in Puerto Rico, *J. Parasitol.* **33:** 253–259.

Fox, I., 1951. Relative and seasonal abundance of the common rat ectoparasites of San Juan, Puerto Rico, *J. Parasitol.* **37:** 85–95.

Mohr, C. O., Good, N. E., and Schubert, J. H., 1953. Status of murine typhus infection in domestic rats in the United States, 1952, and relation to infestation by oriental rat fleas, *Amer. J. Pub. Health* **43:** 1514–1522.

Pomales Lebrón, A., 1948. Studies on murine typhus in Puerto Rico, *Puerto Rico J. Pub. Health & Trop. Med.* **23:** 393–407.

Pomales Lebrón, A., Arbona, G., Morales Otero, P., and Koppisch, E., 1946. The complement-fixation and Weil-Felix reactions in a study of typhus among wild rats, *Puerto Rico J. Pub. Health & Trop. Med.* **21:** 350–356.

Pratt, H. D., and Good, N. E., 1954. Distribution of some common domestic rat ectoparasites in the United States, *J. Parasitol.* **40:** 113–129.

Rickard, E. R., and Riley, E. G., 1948. A state-wide survey of typhus fever in Florida, *Amer. J. Pub. Health* **38:** 541–549.

U. S. Department of Commerce, Weather Bureau, 1954. Local climatological data with comparative data, San Juan, Puerto Rico, 1954, 3 pp.

U. S. Public Health Service, 1952. Trends, *Pub. Health Rep.* **67:** 200.

SOME OBSERVATIONS ON THE BIONOMICS OF THE COMMON MOSQUITOES OF THE NILE DELTA[1]

HERBERT S. HURLBUT AND BERNARD WEITZ[2]

During a study of the epidemiology of West Nile virus infection in the Nile Delta (Taylor *et al.*, 1956), observations were made on the seasonal and geographical distribution, and the feeding habits of the common mosquitoes of the cultivated areas. The present paper is based on data obtained in the course of this study.

SEASONAL DISTRIBUTION

Most of the collections were made in the villages of Barada, Sindbis and Quaranfil about 30 km. north of Cairo, by means of light traps of the New Jersey type (Malhern, 1942) which were operated from sunset to sunrise, usually four nights per week. The power was supplied by 6 volt, 300 amp-hour storage batteries. Each trap was equipped with a 15 watt clear glass bulb and a fan which rotated at a speed of 2,200 rpm. Table 1 gives the data from the operation of these traps for the period April, 1953 to March, 1954. *Culex antennatus*, *C. univittatus*, *C. pipiens*, *Aedes caspius*, and *Anopheles pharoensis* constituted 99.4 per cent of the total catch of 16,362 specimens. *C. poicilipes*, *Culiseta longiareolata*, *Uranotaenia unguiculata*, *Anopheles coustani tenebrosus*, and *A. multicolor* were taken in relatively small numbers. *Culex antennatus* is by far the most abundant species. *C. antennatus*, *C. univittatus* and *A. pharoensis* are warm weather mosquitoes with greatest abundance in July, August, September, and October. *Aedes caspius* also appears to belong in this category. *Culex pipiens* is most abundant in April and May with reduced numbers during July, August, and September and a rise in October and November. This species is present in appreciable numbers during the winter months as indicated by catches in sleeping rooms in rural dwellings (Table 2). Dissections revealed ovaries in all stages of development indicating continuous breeding throughout the winter. This is further supported by finding numerous specimens with recent blood meals and by the presence of males, and of numerous larvae and eggs. No specimens with enlarged fat bodies were found as is observed in hibernating individuals.

Additional observations made in the Barada-Sindbis-Quaranfil area during the summers of 1952 and 1954 were in essential agreement with the records presented in Table 1. Extensive light trap collections made in the Northern Delta near the limits of the cultivated area during the summer of 1954 indicated substantially

[1] This study was conducted under the auspices of the Ministry of Public Health of the Egyptian Government and the U. S. Naval Medical Research Unit No. 3, with aid from the Division of Medicine and Public Health of the Rockefeller Foundation, and latterly from the office of Naval Research through a contract administered by the University of Chicago.

The opinions or assertions contained herein are the private ones of the authors and are not to be construed as official or reflecting the views of the Navy Department or the Naval Service at large.

[2] The Lister Institute of Preventive Medicine, Elstree, Herts, England.

TABLE 1

Monthly catches of female mosquitoes in light traps Barada, Sindbis, Quaranfil, April 1953–March 1954

Month	No. of Trap-nights	Species—No. per Trap-night				
		C. antennatus	*C. univittatus*	*C. pipiens*	*A. caspius*	*A. pharoensis*
Jan.	42	0.00	0.02	0.31	0.00	0.00
Feb.	48	0.00	0.00	0.21	0.00	0.00
March	31	1.58	0.26	2.00	0.19	0.00
April	44	1.52	0.00	1.45	0.07	0.07
May	39	12.15	0.87	4.05	0.74	0.82
June	56	4.11	0.77	2.12	0.41	0.43
July	73	10.57	9.71	1.00	1.30	2.62
August	60	21.46	9.82	0.60	1.28	3.42
Sept.	65	57.72	25.86	0.15	.75	1.14
Oct.	63	15.87	11.94	1.01	1.51	0.51
Nov.	36	20.64	1.50	3.03	1.08	0.25
Dec.	51	0.43	0.00	0.98	0.00	0.02
Totals	608	17.51	6.37	1.26	0.68	0.94

TABLE 2

Mosquitoes collected in sleeping rooms rural dwellings—Nile Delta November 1953–March 1954

Place	No.* rooms	Female *Culex pipiens*						Male *C. pipiens*
		Total no.	Stomach content		Ovaries			
			Non-nucl. RBC	Nucl. RBC	Undeveloped	Developing	Mature	
Mit Abu Ghalib	62	61	25	0	25	9	4	6
Ezbet Bisintawi	45	2	2	0	1	1	0	0
Ezbet Chenouda	65	15	13	1	11	1	0	3
Sindbis Health Dist	145	213	176	6	93	46	19	10
Totals	317	291	216	7	130	57	23	19

* 10–50 rooms each month at each place.

lower mosquito densities compared with the Barada-Sindbis-Quaranfil area, and the peak abundance of *C. antennatus* and *A. pharoensis* tended to come somewhat earlier. These records are presented in Table 3.

The relative frequencies by seasons of the five common species in larval collections made in the Barada-Sindbis-Quaranfil area are given in Table 4. Eggs of *C. antennatus* and larvae and pupae of *C. univittatus* and *A. pharoensis*, in small numbers, were collected in early February. These data support and are in general agreement with the seasonal records for adult mosquitoes taken in light traps (Table 1).

TABLE 3

Monthly catches of female mosquitoes in light traps, Nile Delta, 1954

Place	Month	No. of Trap-Nights	Species—Specimens per Trap-Night						
			CA	CP	CU	AC	AP	ACO	CPO
Mit Abu Ghalib	June	18	8.66	0.81	0.11	0.39	1.94	0.00	0.00
	July	27	11.15	0.22	0.00	0.15	8.82	0.00	0.07
	Aug.	30	3.36	0.07	0.00	0.10	2.04	0.20	0.07
	Sept.	28	1.86	0.57	0.00	0.07	0.36	0.44	0.04
	Oct.	24	4.08	0.42	0.00	0.00	0.29	0.12	0.25
	Nov.	20	0.50	0.45	0.00	0.15	0.25	0.00	0.10
Ezbet Bisintawai	June	28	40.51	0.36	0.25	1.11	0.43	0.00	0.07
	July	28	15.45	0.14	0.00	0.54	2.13	0.00	0.21
	Aug.	28	26.62	0.07	0.00	0.43	2.82	1.32	1.18
	Sept.	30	2.44	0.30	0.00	0.13	0.50	4.86	0.77
	Oct.	38	2.08	0.55	0.03	0.11	0.08	0.47	0.24
	Nov.	14	0.21	0.07	0.00	0.00	0.00	0.00	0.07
Kafr Mahallet Dawud	June	28	107.64	0.71	0.14	5.46	4.39	0.00	0.00
	July	27	142.93	0.07	0.04	3.00	5.29	0.00	0.04
	Aug.	30	21.50	0.03	0.00	0.63	7.13	0.00	0.10
	Sept.	26	21.82	0.08	0.04	0.62	2.00	0.65	0.77
	Oct.	37	3.94	0.30	0.03	0.24	0.41	0.08	0.75
	Nov.	12	1.92	0.08	0.00	0.17	0.00	0.00	0.42
Ezbet Chenouda	June	22	11.43	1.77	0.23	7.36	0.14	0.00	0.00
	July	32	12.50	0.19	0.22	19.81	6.22	0.03	0.03
	Aug.	28	8.97	0.14	0.14	6.35	4.93	0.04	0.17
	Sept.	26	10.85	0.08	0.15	3.04	3.23	1.96	0.50
	Oct.	32	1.97	0.37	0.47	1.94	0.22	2.12	0.31
	Nov.	22	1.32	0.05	0.09	1.00	0.05	0.00	0.14
Barada	June	25	13.68	11.31	1.40	1.16	1.16	0.04	0.08
	July	30	49.73	2.43	4.20	8.53	5.96	0.00	0.10
	Aug.	22	154.09	2.14	14.22	4.09	15.92	0.09	0.41
	Sept.	23	284.35	2.34	61.04	1.78	0.87	0.78	0.22
	Oct.	27	230.96	11.67	25.48	0.82	1.48	0.22	0.63
	Nov.	25	61.75	14.60	2.00	0.20	0.72	0.16	0.28
Delta Barrage	June	10	4.80	2.80	0.70	0.70	0.00	0.00	0.00
	July	14	9.22	2.36	5.73	2.28	1.00	0.00	0.00
	Aug.	15	21.87	1.67	10.87	7.07	0.73	0.00	0.07
	Sept.	16	176.31	5.18	98.81	17.81	7.76	1.56	0.44
	Oct.	14	85.00	23.20	53.40	9.85	1.86	1.50	3.28
	Nov.	14	11.63	11.70	13.85	4.79	0.14	0.14	2.07

CA = *Culex antennatus* CP = *Culex pipiens* CU = *Culex univittatus* AC = *Aedes caspius* AP = *Anopheles pharoensis* ACO = *Anopheles coustani* CPO = *Culex poicilipes*

GEOGRAPHICAL DISTRIBUTION

As already mentioned mosquitoes were extensively collected in the northern part of the Nile Delta by means of light traps during the summer of 1954. These collections indicate that *C. univittatus* is relatively much less abundant in the

TABLE 4

Seasonal collections of mosquito larvae Sindbis Health District, 1953

Season	No. of Collections	Percentage of Collections Containing Each of the Five Common* Species				
		Culex antennatus	*Culex univittatus*	*Culex pipiens*	*Aedes caspius*	*Anopheles pharoensis*
Jan.† Feb. March	26	38	35	73	27	8
April, May, June	26	35	35	58	12	12
July, Aug. Sept.	31	75	84	13	13	71
Oct. Nov. Dec.	40	47	50	67	7	17
Total	123	50	52	53	14	28

* More than 95 per cent of the 4303 specimens identified.
† January 1954.

TABLE 5

Light trap catches of female mosquitoes Nile Delta, June–November, 1954

Locality	No. Trap-Nights	Total Catch	Mosq. per Trap-Night	Per cent—Species					
				CA	CP	CU	AC	AP	Other† Species
Northern Delta*	473	13,429	28	85.20	1.11	0.13	2.71	7.98	2.87
Ezbet Chenouda	162	3,099	19	41.24	2.07	1.19	36.66	13.94	4.87
Barada-Barrage	235	33,396	142	72.53	5.35	15.84	3.23	2.43	0.62

CA = *Culex antennatus* CP = *Culex pipiens* CU = *Culex univittatus* AC = *Aedes caspius* AP = *Anopheles pharoensis.*

* Mit Abu Ghalib, Ezbet Bisintawai, Kafr Mahallet Dawud.

† *Anopheles coustani tenebrosus, Culex poicilipes, Culiseta longiareolata, Uranotaenia unguiculata.*

north while it comprised about 15 per cent of the total collection in the area 30 km. north of Cairo at the southern tip of the Delta. *A. coustani tenebrosus* and *C. poicilipes* proved to be relatively more numerous in the northern part of the Delta. Table 5 gives the relative frequencies of the different species collected.

The locations sampled in the north were, Mit Abu Ghalib on the Damietta branch of the Nile about 20 km. south of Damietta, Ezbet Bisintawai about 12 km. north of Damanhur, Kafr Mahallet Dawud 5 km. southwest of Dusuq and Ezbet Chenouda about 10 km. southeast of Kafr El Sheikh.

FEEDING HABITS

About five per cent of the specimens caught in light traps contained blood. The sources of these bloods were determined by precipitin tests using the techniques described by Weitz (1952). The feeds were tested with absorbed antisera prepared against the sera of man, ox, sheep or goat, horse donkey or mule, dog and lastly camel. No cross reactions occurred when these anti-sera were tested with the sera derived from heterologous species except for the group reactions of sheep

TABLE 6

Mosquito stomach blood contents identified by precipitin test. Mosquitoes caught in light traps except C. pipiens *Sample # 2 which came from bedrooms. Barada-Sindbis-Quaranfil Area*

Mosquito	Percentages									Number Negative Tests	Total Blood Sources* Determined
	Man	Ox	Bovine (not ox)	Sheep Goat	Camel	Dog	Horse Donkey Mule	Mammalian unidentified	Avian		
C. antennatus #1	22.3	9.3	No test	0.8	0	5.4	32.3	28.4	1.5	4	130
C. antennatus #2	17.1	36.2	17.2	0.6	0	4.5	22.2	2.2	0.0	73	315
C. univittatus	11.3	5.0	No test	1.2	1.2	12.5	11.3	48.7	8.8	8	80
C. pipiens #1	95.3	1.2	No test	0	0	0.3	1.2	2.0	0.0	5	253
C. pipiens #2	95.7	0.9	No test	0	0	0.4	0.7	1.7	0.6	147	1066

* About 4 per cent of the mosquito stomachs contained blood from two sources. These sources are counted individually.

and goat sera with anti-sheep serum, and the reactions with horse, donkey and mule sera which occurred with anti-horse serum. No attempt was made to distinguish these species. When no reactions were obtained with any of these anti-sera the blood meal was tested with anti-mammalian and anti-avian sera, known to react with the serum of all the possible mammalian or avian hosts respectively. Failure to obtain positive reactions with these anti-sera indicated that the blood meal was not suitable for testing and the results were recorded as negative feeds. The results are presented in Table 6.

It was originally assumed that the anti-ox serum would give cross reactions with the Egyptian variety of the Indian water buffalo but when serum of the water buffalo was available it was found that it reacted only at a low titer with anti-ox. It seems likely that most of the bloods grouped under the "mammalian, unidentified" heading for *C. antennatus* sample No. 1 and *C. univitattus*, in Table 6, are from water buffalo as tests were made for all other possible mammalian hosts with negative results, although the reactions with anti-mammalian serum indicated that the blood meal extract was sufficiently concentrated. This is supported by the results presented in Table 6 for *C. antennatus* No. 2 which were additionally tested with an anti-bovine serum known to react with water buffalo serum at a high titer. This test was applied to all the meals of this group which reacted with anti-mammalian serum but failed to react with the other anti-sera. It will be noted that 17 per cent of the bloods reacted with this anti-bovine serum and only 2 per cent remained in the unidentified mammal category.

As already stated, the light traps were operated in typical rural villages from sunset to sunrise. Thus, the samples may perhaps be taken as representative of this particular environment. It will be noted that *C. antennatus* fed most commonly on the large mammals and relatively infrequently on birds. *C. univittatus* showed the greatest diversity in its feeding habits, in spite of the small sample, with a relatively larger number of bloods from avian sources. *C. pipiens* from light traps yielded 95 per cent human blood, and a much larger sample from

TABLE 7

Female mosquitoes caught in traps, Barada September 1–October 14, 1954. 26 nights

Type Trap	Total Catch	Percentage of Species Caught					
		Culex antennatus	*Culex pipiens*	*Culex univittatus*	*Aedes caspius*	*Anopheles pharoensis*	*Other* species*
Light	11,365	84	0.9	14	0.4	0.3	0.4
Bait-man	1,206	51	27	15	1	5	1
Percentage in bait trap with blood in stomach		29	88	45	65	77	50
Percentage human blood by precipitin test		81.5	100	92	—	97	—

* *Anopheles coustani, Culex poicilpes.*

bedrooms gave an almost identical distribution. It appears that *C. pipiens* from this source is highly anthropophilic and seldom feeds on avian blood.

During September and October 1954, a trap modeled after the Bates portable stable trap (Bates, 1944) was operated at Barada for 26 nights from sunset to sunrise, with a man serving as bait. A light trap was operated simultaneously in the same neighborhood at a distance sufficient to prevent interference. The same five common species were taken in both traps, with *C. antennatus* predominating. Precipitin tests showed that over 80 per cent of the stomach bloods were from a human source. Relatively larger numbers of *C. pipiens* and *A. pharoensis* were caught in the man-baited trap. Also, a higher percentage of these two species contained blood. *C. univittatus* was caught in about the same proportion in each trap and 45 per cent of those in the man-baited trap had blood in the stomach. The data are presented in Table 7.

A smaller trap of similar design baited with a buff-backed heron and operated for 23 nights during the period July–October, 1953 caught 1 *C. antennatus*, 127 *C. univittatus*, and 3 *C. pipiens*. Sixty-nine per cent of the *C. univittatus* had taken blood which precipitin tests proved to be avian. Light traps operated simultaneously yielded these species in about the proportion indicated in Table 1, with *C. antennatus* greatly predominating. Thus *C. univittatus* showed relative preference for avian blood.

Mosquito collections were also made by catching specimens for about two hours after sunset while they were biting man. The data from nine such catches

TABLE 8

Mosquitoes caught while biting man 3 localities in the Nile Delta*

Number of Collections	Number of Mosquitoes Caught				
	C. antennatus	*C. univittatus*	*C. pipiens*	*A. caspius*	*A. pharoensis*
9	142	3	9	47	128

* Sindbis, Mit Abu Ghalib, Ezbet Chenouda. July–September.

from three localities are presented in Table 8. A few observations were also made on farm animals. *C. antennatus*, *A. caspius* and *A. pharoensis* were observed biting the dairy cow, donkey, and water buffalo.

OTHER OBSERVATIONS

C. univittatus was colonized and maintained in the laboratory for over two years. It mates readily in a cylindrical cage 30 cm. in diameter by 40 cm. high. The ordinary laboratory albino mouse was satisfactory as a blood source. Autogenous eggs were not observed. The larval food consisted of a broth culture of a strain of *Aerobacter aerogenes* (M-14) isolated from a natural breeding place, which was added to Loeffler's blood serum in the proportion of 200 ml. of the bacterial culture to 300 ml. of Loeffler's blood serum. Fifteen ml. of this mixture was added daily to larval pans containing 250 larvae and 3 liters of water. The regular addition of a pure culture of *A. aerogenes* was found to be superior to reliance upon chance contamination. Rozeboom (1935) observed that this organism was of some value as larval food. The egg stage of *C. univittatus* requires 24 hours and the pupal stage 30 hours at 30°C. Adults emerge 7–9 days after eggs are deposited, at this temperature.

C. antennatus larvae were reared using the same technique. The length of the egg, larval, and pupal stages was approximately the same as for *C. univittatus* at 30°C. *C. antennatus* did not mate in captivity; for this reason we failed to establish a laboratory colony.

C. pipiens was colonized and maintained by the same technique described for *C. univittatus*. The aquatic stages require 8–10 days at 30°C., about one day longer than the other two species.

Knight and Abdel Malek (1951) found that a small percentage of the female *pipiens* from suburban Cairo were capable of producing autogenous eggs. During the present study, freshly engorged females were collected in bedrooms in Barada, a typical rural village 30 km. north of Cairo. These were brought into the laboratory and allowed to deposit eggs. Nine egg rafts obtained from them were segregated and the larvae reared separately. The resulting adults were confined in one quart glass fruit jars containing about 100 ml. of water and lined with moist filter paper. The top was covered with netting. Five per cent dextrose was given as the only food. Two viable autogenous egg rafts were obtained from one of the nine groups. After thirty days, the 119 surviving females from all the groups were dissected. None of the ovaries showed development. Thus, of nine specimens engorged with blood, taken in a typical rural environment, one had offspring capable of producing viable autogenous eggs.

C. pipiens in the Nile Delta lives in intimate association with man and is strongly anthropophilic. As many as 515 were taken in a single bedroom on one occasion in late April and 502 of them were engorged with blood. It was the only species found in large numbers in a diurnal resting place. As already stated, this mosquito is most numerous in April and May, declining during the hot months of July, August, and September and increasing again in October and November. The larvae are often found in large numbers in the effluent from cesspits about mosques and villas and in pools of water near collections of manure and compost.

SUMMARY

Culex antennatus, *C. univittatus*, *C. pipiens*, *Aedes caspius*, and *Anopheles pharoensis* were found to be the most common mosquitoes of the cultivated areas of the Nile Delta. This finding is based on collections of adults from light traps, baited traps, and diurnal resting places, and from larval collections. *C. antennatus*, *C. univittatus*, *A. pharoensis*, and *A. caspius* are warm weather mosquitoes with greatest abundance in July, August, September and October. *C. antennatus* occurs in by far the greatest numbers. *C. pipiens* is most abundant in April and May with reduced numbers during July, August, and September and a rise in October and November. It is present and actively breeding in appreciable numbers during the winter months. *C. univittatus* is a common mosquito in the southern part of the Nile Delta but tends to become relatively less abundant as the northern limit of the cultivated area is approached.

Each of the five common species feeds readily on man. *C. pipiens* and *A. pharoensis* appear to be most strongly anthropophilic. Of the three species of *Culex*, *C. univittatus* is probably the most diverse in its feeding habits, and is most strongly attracted to birds. *C. antennatus* seems to feed most commonly on man and the large domestic mammals.

C. univittatus was colonized without difficulty and the colony was maintained for over two years. *C. antennatus* failed to mate in captivity, and consequently was not colonized.

ACKNOWLEDGMENTS

The authors are indebted to CDR. K. L. Knight MSC USN, Bureau of Medicine and Surgery, Navy Department, Washington, D. C. for assistance in mosquito taxonomy and Lt. C. D. McGuire MSC USN, Department of Bacteriology, NAMRU-3, for the identification of the organism used as larval food. They also express their appreciation for the technical assistance rendered by E. W. Spangler, HMC USN, H. R. Fallers, Jr., HM1 USN, M. Hassanein, and Saif El Nasr Shehata.

REFERENCES

BATES, M., 1944. Notes on the construction and use of stable traps for mosquito studies, *J. Nat. Mal. Soc.* **3**: 135.

KNIGHT, K. L., AND ABDEL MALEK, A. A., 1951. A morphological and biological study of *Culex pipiens* in the Cairo area of Egypt, *Bull. Soc. Fouad Ier Entom. XXXV;* 175–185.

MALHERN, T. D., 1942. New Jersey mechanical trap for mosquito surveys, *N. J. Agric. Sta. Circ.* No. 420.

ROZEBOOM, L. E., 1935. The relation of bacteria and bacterial filtrates to the development of mosquito larvae, *Amer. J. Hyg.* **21**: 167–179.

TAYLOR, R. M., WORK, T. H., HURLBUT, H. S., AND RIZK, F., 1956. A study of the ecology of West Nile virus in Egypt, *Am. J. Trop. Med. & Hyg.* **5**: 579–620.

WEITZ, B., 1952. The antigenicity of sera of man and animals in relation to the preparation of specific precipitating antisera, *J. Hyg., Camb.* **50**: 276–294.

THE INFLUENCE OF MATING ON EGG PRODUCTION BY *AEDES AEGYPTI*

CALVIN A. LANG[1]

Department of Entomology, The Connecticut Agricultural Experiment Station, New Haven 4, Connecticut

The necessity of a blood meal for egg production by adult female mosquitoes of certain species is an established and unique phenomenon of mosquito biology (Putnam and Shannon, 1934), and the term, Blood Meal Factor or BMF, can be applied to describe the unidentified active principle(s) in the blood. In this investigation of BMF the initial efforts resulted in an artificial feeding procedure using a sucrose supplement to stimulate engorgement of the female mosquitoes on otherwise unattractive test samples (Lang and Wallis, 1956). Also some of the factors affecting egg production, such as non-optimal oviposition sites (Wallis, 1954; Woke, 1955) and insufficient blood meal intakes (Roy, 1936; Woke, unpublished data) were considered. Another factor that was questioned was the role of mating, for the results of preliminary experiments indicated a possible relationship between the presence of males and maximal egg deposition. A more extensive study, therefore, was undertaken in which the feeding propensities, eggs/mosquito ratios, and oviposition times of virgin and mated mosquitoes were determined. The results of these experiments are presented in this communication.

MATERIALS AND METHODS

In this investigation the species of mosquito employed was a strain of *Aedes aegypti* obtained originally from the Johns Hopkins School of Hygiene and Public Health insectary and maintained at this institution for the past two years. The larvae were reared in shallow, white enameled, photographic trays (48 x 35 x 5 cm.) containing a medium of tap water and commercial rabbit feed pellets. The room temperature varied from 24–28°C., and no attempts were made to control the lighting conditions in the rearing room, which faced north and received supplementary illumination during the working hours from overhead fluorescent lights. After nine to ten days, the larvae pupated and were sexed and individually isolated to prevent contact between the sexes.

The adult mosquitoes which emerged during a single 24-hour period were distributed into 3 cages, which consisted of 1 quart, square Mason jars (8.0 x 8.0 x 14 cm.) with a cover of black, nylon marquisette netting (40 mesh) secured with a ring-type lid. The first conditioning cage contained 10–15 females; the second, 10–15 females and twice that number of males; and the third, 10–15 male mosquitoes. Water was added to each cage to a depth of about 2 cm. to raise the humidity. A pad of absorbent cotton saturated with 10 per cent sucrose

[1] Present address: Division of Medical Entomology, Johns Hopkins School of Hygiene and Public Health, Baltimore 5, Maryland.

solution was placed on top of the netting of each cage to furnish food. Unless otherwise stated, this was the feeding procedure used throughout these experiments. After 3 to 4 days of conditioning, the mosquitoes were transferred with an aspirator to dry cages and deprived of food and water for 20–24 hours. The male mosquitoes then were removed from the second cage containing both sexes and discarded. The females in both the first and second cages were offered a blood meal by placing the forearms of a single human host on top of the net cover for 10 minutes, and the feeding responses were noted.

Following this meal, the individual engorged females and, when required, virgin males from the third stock cage, were transferred to the test cages. These enclosures were similar to the others except that the bottom and part of two sides were lined with a single sheet of wet Whatman No. 3 filter paper (6.5 x 15 cm.) to serve as an oviposition site, and distilled water was added to a depth of about 1 cm. The insects were distributed in the test cages as follows:

The females from the first stock cage were placed in Group 1 (virgin females) and Group 3 (post-blood meal mating), and the females from the second cage were allocated to Group 2 (pre-blood meal mating) and Group 4 (pre- and post-blood meal mating). Each cage contained a single female, and those in Groups 3 and 4 also included two virgin males.			

The term, mating, as used in this paper is defined as the cohabitation of females and males.

For the next 14 days following the blood meal, the test cages were kept in a cabinet with glass doors, in which the temperature varied from 24–30°C. during the overall 3 month experimental period. Illumination was provided by daylight fluorescent lamps for 8–10 hours/day, and the maximum light intensity at the experimental cages was 60–70 foot candles. During the entire post blood meal test period the cages were examined each morning and late afternoon to determine oviposition times. On the 7th day of this period the mosquitoes which had laid eggs were removed and pooled according to their respective groups, and the number of eggs deposited was determined. The counting was performed with the aid of a hand tally, and the recorded figure was an average of two successive counts. In some trials the egg viability was determined by immersing the eggs in water and counting the number of resultant first instar larvae. During the next 7 days the nonlaying and pooled mosquitoes were maintained as before, and on the 14th day following the blood meal, the eggs in these remaining cages were counted. This experimental procedure was followed 5 times using insects from a different larval hatch for each trial, so that a total number of 129 female mosquitoes was studied.

RESULTS

Virtually all of the females in this study fed to some extent when offered a blood meal, and a large number of these became engorged (Table 1). It will be observed that the two groups responded similarly, for 86 per cent of the virgins fed to repletion as compared to 88 per cent of the mated females.

The data on egg deposition 7 days after the blood meal are presented in Table

TABLE 1

The arm-feeding response of 4–5 day old experimental Aedes aegypti

Group	No. Trials	No. Mosquitoes	Per cent Engorging	
			Mean ± S.E.	Range
Virgin females	5	69	86.2 ± 4.2	75–94
Mated females	5	60	87.6 ± 5.3	73–100

TABLE 2

The eggs/mosquito ratios of individual female Aedes aegypti *7 days post-blood meal*

Group	Description	Observed Data				Adjusted Data		
		No. Mosquitoes	Eggs/Mosquito		No. Non-laying Mosquitoes	No. Normal Mosquitoes	Eggs/Mosquito	
			Mean ± S.E.	Range			Mean ± S.E.	Range
1	No mating	30	54.1 ± 7.97	0–118	9	18	86.8 ± 4.41	45–118
2	Pre-blood meal mating	25	70.3 ± 6.40	0–119	2	21	82.1 ± 3.58	47–119
3	Post-blood meal mating	25	64.5 ± 6.84	0–106	3	20	78.3 ± 4.70	39–106
4	Pre- and post-BM mating	26	86.4 ± 3.86	35–116	0	25	88.4 ± 3.42	56–116

2. The largest number of eggs per mosquito was deposited by the females in Group 4, which were caged with males during their entire adult life. The average ratio of Group 4, 86.4 eggs/mosquito, was greater statistically when analysed by a ranking method (Wilcoxon, 1945) than the ratios of Group 2 ($P < .05$), Group 3 ($P < .05$) and Group 1, the virgin females ($P < .01$). In addition, the standard error, a criterion of both the individual variation and experimental errors, was lowest in Group 4.

Upon inspecting these data it appeared that a large proportion of the virgin and partially mated females laid no eggs, whereas all of the Group 4 mosquitoes oviposited. The above figures were adjusted, therefore, as follows: the data were regarded for the sake of convenience as being derived from a normal frequency distribution, because these enumeration values did not fit a Poisson distribution. On this basis the 98 per cent interval of the Group 4 ratios, which were maximal, was estimated using the formula, arithmetic mean ± 2½ standard deviations, and found to be 37 to 118 eggs/mosquito, i.e. the probability of a mosquito from the same population laying a number of eggs outside that interval was less than 2 times in a hundred. Values of all groups falling outside this range were considered abnormal and discarded, and the data on the remaining normal females were recalculated. It was observed that not only did the differences between the means disappear, but that the standard errors of all groups were now equal in magnitude and comparable to the Group 4 unadjusted figure.

An important aspect of an assay procedure for BMF is the length of the time period following the blood meal necessary to permit maximal oviposition. Since

TABLE 3

The oviposition rate of experimental Aedes aegypti

Group	Description	No. Eggs	Per cent No. of Eggs Laid	
			0–7 days	8–14 days
1	No mating	2169	59	41
2	Pre-blood meal mating	1882	93	7
3	Post-blood meal mating	1754	93	7
4	Pre- and post-BM mating	2322	97	3

oviposition first was observed 3–5 days after the blood meal, the eggs were counted arbitrarily on the 7th and on the 14th day. The results are presented in Table 3. Even though a similar number of eggs was produced by each ovipositing female, regardless of her mating condition, it was noted that only 59 per cent of the "virgin" eggs were deposited during the first 7 days. On the other hand 93 per cent of the eggs of Group 2 and 3 and 97 per cent of the eggs of Group 4 were laid during this same period. These data, therefore, indicate that the oviposition times of mated mosquitoes were shorter than that of virgin females.

DISCUSSION

Earlier in this communication the term "mating", as applied in these experiments, was defined as the exposure to mating conditions rather than the copulatory act itself. To support this definition with biological instead of etymological evidence, some of the eggs were tested for viability. As other studies have demonstrated, all the eggs laid by the virgin mosquitoes were non-viable, whereas a large proportion of the eggs deposited by the partially and continuously mated females were fertile and hatched. In these studies, therefore, the egg hatchability data provided clear evidence of successful mating. This determination can be considered a better criterion of the overall mating process than the presence of spermatozoa or copulation behavior, for these latter methods must rely ultimately on hatchability tests for their validity in order to eliminate the possibilities of sterile and inadequate numbers of spermatozoa.

Certain experimental conditions are necessary and optimal for mating and oviposition. Roth (1948) has shown that temperatures of 25–27°C. are preferred, for the males are more active in this range. In the same study, male *A. aegypti*, unlike *Anopheles quadrimaculatus* and *Culex pipiens*, were observed to be in a continual mating state as soon as their genitalia rotated and their antennal fibrillae extended. Seaton and Lumsden (1941) demonstrated in their investigation of *A. aegypti* that over 80 per cent of a laboratory population of virgin females became inseminated 3 days after emergence and the largest percentage of females fed between the 3rd and 5th day of adult life. All of these conditions were incorporated into the present experimental procedure.

In a study of the laboratory mating habits of *A. quadrimaculatus*, it was concluded that inseminated females are more blood-avid than virgin females (Stahler and Terzian, 1953). However, in the present study, no differences in the

engorgement rates of *A. aegypti* were observed, which substantiate the findings of Seaton and Lumsden (1941). Although this discrepancy in feeding behavior can be attributed to species differences, the age and conditioning of the insect preparations also may be involved.

In these experiments the virgin females produced the fewest eggs per mosquito and the continuously mated mosquitoes, the most. The response of this latter group was regarded as optimal and normal, since every female in this group oviposited and since in nature, females are continuously exposed to males. A statistical evaluation of this response resulted in a rejection criterion which was applied to all the data. The adjusted data indicated that the conspicuous dissimilarities between various groups were due to the large number of virgin and partially mated females which laid few or no eggs. Since several females usually are included in a single cage in blood meal studies, any non-laying individuals would alter significantly the average eggs/mosquito ratio. These findings demonstrate the necessity of exposing the females continuously to males in order to insure maximal egg deposition under these experimental conditions.

Two strains of *A. aegypti* recently have been reported which differ in ovulation behavior (Gillett, 1955). In the Newala strain a much higher percentage of the mated females oviposited than of the unmated females, whereas in the Lagos strain the behavior of both groups was similar. Additional evidence indicated that the mating act itself did not stimulate oviposition by the virgin mosquitoes. These findings are particularly applicable and appropriate to the present study.

Another objective of this investigation was to obtain an estimate of the expected eggs/mosquito ratio under our laboratory conditions. This information was necessary to establish rejection standards for use in future studies of BMF. According to the present data the average ratio should fall within the interval, 79.9–97.0, which is equivalent to the mean $\pm$ 2½ standard errors of the Group 4, adjusted data (see Table 2). The mean values of individual female *A. aegypti* under several other conditions ranged from 87–98 and, therefore, are in agreement with this statistic (Woke, 1937; Woke *et al.*, 1956).

Since the only group in which all the females oviposited had continuously mated, and since approximately ⅓ of the virgin females failed to oviposit, it is logical to assume that a non-laying, engorged female has not mated successfully. Based on this assumption, it can be concluded tentatively that the fed and unfed females in this study had equal chances of being mated, for a similar percentage of each group laid eggs. These data support and disagree, respectively with those of others (Stahler and Terzian, 1953; Roth 1948). Additional experimentation, which should include spermathecal examinations or egg viability tests, would be required to obtain unequivocal results on the mating rates of unfed and engorged females.

An examination of the data collected on oviposition times of the various groups disclosed another difference. With partial or continuous mating conditions a post-blood meal period of 7 days was observed to be sufficient time for maximal oviposition. In fact, use of a longer period was inadvisable, for hatching occurred and thereby complicated the egg counting procedure. During this 7 day interval,

however, only 60 per cent of the eggs of the virgins were deposited. This difference also has been observed by Gillett (1955) who suggested that impregnation by the male shortens the oviposition time. The physiology of this phenomenon would be a fascinating investigation.

SUMMARY

The egg production of individual virgin and mated female *Aedes aegypti* was investigated under laboratory conditions. No differences in the engorgement rates were observed. However, the continuously mated females deposited a statistically and significantly greater number of eggs per mosquito than did the virgin and partially mated females. These differences were attributable to the large proportion of females in the latter groups which laid few or no eggs. In these experiments, therefore, almost all ovipositing females, regardless of their mating conditioning, laid approximately the same number of eggs. An additional finding of this study was the delayed oviposition times of virgin mosquitoes.

ACKNOWLEDGMENT

The cooperation of my colleague, Dr. Robert C. Wallis, in furnishing the mosquitoes is gratefully acknowledged.

REFERENCES

GILLETT, J. D., 1955. Behavior differences in two strains of *Aedes aegypti*, *Nature* **176:** 124–125.

LANG, CALVIN A., AND WALLIS, ROBERT C., 1956. An artificial feeding procedure for *Aedes aegypti*, L. using sucrose as a stimulant, *Am. J. Trop. Med. & Hyg.* **5:** 915–920.

PUTNAM, P., AND SHANNON, R. C., 1934. The biology of *Stegomyia* under laboratory conditions. II. Egg-laying capacity and longevity of adults, *Proc. Ent. Soc. Wash.* **36:** 217–242.

ROTH, LOUIS M., 1948. A study of mosquito behavior. An experimental laboratory study of the sexual behavior of *Aedes aegypti* (Linnaeus), *Am. Midland Naturalist* **40:** 265–352.

ROY, D. N., 1936. On the role of blood in ovulation in *Aedes aegypti*, Linn., *Bull. Ent. Res.* **27:** 423–429.

SEATON, D. R., AND LUNSDEN, W. H. R., 1941. Observations on the effects of age, fertilization, and light on biting by *Aedes aegypti* (L.) in a controlled microclimate, *Ann. Trop. Med. Parasit.* **35:** 23–36.

STAHLER, N., AND TERZIAN, L. A., 1953. Studies in the laboratory mating habits of *Anopheles quadrimaculatus*, Say, *J. Exp. Zool.* **124:** 317–328.

WALLIS, ROBERT C., 1954. The effect of population density and of NaCl concentrations in test series in laboratory experiments with ovipositing *Aedes aegypti*, *Mosquito News* **14:** 200–204.

WILCOXON, F., 1945. Individual comparisons by ranking methods, *Biometrics Bull.* **1:** 80–82.

WOKE, PAUL A., 1937. *Comparative Value of Blood and Blood Fractions from Different Species of Vertebrates in the Nutrition of Adult Mosquitoes*, Doctoral dissertation, School of Hygiene and Public Health, Johns Hopkins University, Baltimore, Maryland.

WOKE, PAUL A., 1955. Deferred oviposition in *Aedes aegypti* (Linn.), *Ann. Ent. Soc. Amer.* **48:** 39–46.

WOKE, PAUL A., ALLY, M. S., AND ROSENBERGER, C. R., JR., 1956. The numbers of eggs developed related to the quantities of blood ingested in *Aedes aegypti* (Linnaeus). *Ann. Ent. Soc. Amer.* (in press).

AN ARTIFICIAL FEEDING PROCEDURE FOR *AEDES AEGYPTI* USING SUCROSE AS A STIMULANT

CALVIN A. LANG[1] AND ROBERT C. WALLIS

Department of Entomology, The Connecticut Agricultural Experiment Station, New Haven 4, Connecticut

One of the chief difficulties in the investigation of the blood meal phenomenon is to induce mosquitoes to feed on test preparations. Some of the conventional methods have employed thin membranes, warmed test solutions, or erythrocyte suspensions (Woke, 1937; Greenberg, 1951), but these devices were considered too elaborate technically for a routine procedure. A more suitable method using various sugar supplements in the test diet as feeding stimuli has been reported (Dimond *et al.*, 1955; Knierim *et al.*, 1955).

Since our preliminary experiments indicated that some of the naturally occurring sugars are not equally effective in stimulating engorgement, it was deemed advisable to examine this problem more extensively. The preferred sugar of the yellow fever mosquito, *Aedes aegypti*, was determined and incorporated in an artificial feeding procedure as a chemical stimulus. These experiments are described in this communication.

MATERIALS AND METHODS

The larvae of a laboratory culture of *Aedes aegypti*, (originally obtained from the Division of Medical Entomology, Johns Hopkins School of Hygiene and Public Health) were reared at room temperature (22–26°C.) in enamel pans (48 x 33 x 5 cm.) containing a medium of tap water and commercial rabbit feed pellets. After 9–10 days the larvae pupated and were placed in containers of tap water covered with glass lantern chimney cages. The adults which emerged during a single 24-hour period were isolated, transferred to dry cages, and offered the following dietary regimen:

Adult age	*Regimen*
1st–3rd day	Water or 10% glucose solution
3rd–4th day	No liquids
4th day	Test preparations

The maintenance liquids were administered by placing a cotton pad saturated with the solution on the net cover of the cage and allowing the mosquitoes to feed ad libitum for the first three days. During the next 20–24 hours fluids were withheld in order to dehydrate the insects prior to testing.

For each test approximately ten female mosquitoes were transferred from the conditioning cages to each of several one-quart, square Mason jars (8.0 x 8.0 x 14 cm.) covered with black nylon marquisette netting (40 mesh), which was

[1] Present address: Division of Medical Entomology, Johns Hopkins School of Hygiene and Public Health, Baltimore 5, Maryland.

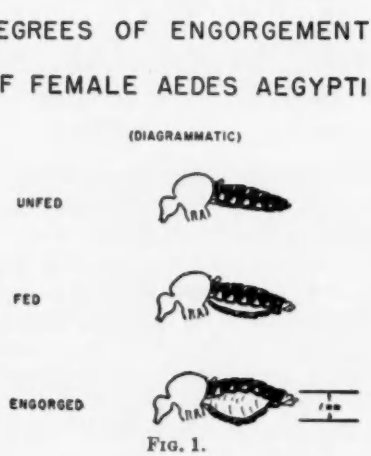


FIG. 1.

secured with a threaded, ring-type lid. A series of sugar solutions was prepared together with a solution of glycerin, for this sugar alcohol has a sweetness index (to man) similar to sucrose. These preparations were administered by saturating a Pyrex wool pad (approximately 2.5 x 5.0 x 1 cm.) with a test solution at room temperature and placing it on top of the netting. The testing area was illuminated from above by fluorescent lights. At the beginning of the 40-minute feeding period the mosquitoes were agitated by blowing into and shaking the jars. Following this period the females were removed with an aspirator, examined macroscopically, and classified as to the extent of feeding. These tests were performed 3 times using a total of 29 $\pm$ 2 mosquitoes per solution. The same general procedure was followed to assay sugar supplemented preparations.

The classification procedure was based on the degree of abdominal distention as measured by the amount of pleural membrane appearing between the tergites and sternites of the second to sixth abdominal segments. (Fig. 1). When no lateral split exposing the pleura was apparent, the insect was termed "unfed"; a split less than 0.5 mm., "fed"; and a split greater than 1.0 mm., "engorged". The "engorged" specimens also exhibited marked abdominal distention when examined from the dorsal aspect. In order to correlate the degrees of engorgement with actual meal weights, the following technique was devised: a single female mosquito was placed into a fine mesh nylon envelope with a draw string opening and weighed on a torsion balance (range 0–50 mg., sensitivity 0.02 mg.). The difference in weights before and after feeding was regarded as the weight of the meal.

When egg deposition was determined, only engorged mosquitoes were used. After feeding, these specimens together with an equal number of males were placed into another Mason jar containing water or 0.125 per cent NaCl solution to provide an optimal oviposition site (Wallis, 1954). The bottom and two sides of the jar were lined with a strip of Whatman #3 filter paper (6.5 x 15 cm.) to

serve as the oviposition site. A 10 per cent glucose solution was offered as the maintenance diet, and seven days later the number of eggs deposited was noted according to the procedure described elsewhere (Lang, 1956).

All sugar solutions were prepared using reagent grade chemicals and distilled water in the following manner: 10.0 g. sugar was dissolved in water, and the solution was made up to a final volume of 100 ml.; the resultant concentration was thus 10 per cent (w/v). To prepare the 5 per cent sugar solutions a given volume of 10 per cent solution was diluted with an equal volume of water. The same formulations were used throughout this study and were stored in a frozen state to minimize microbial growth.

Viscosity measurements were performed at 25.5°C. with Ostwald-Fenske viscosimeters that previously had been calibrated with water. The densities were determined by weighing a measured aliquot on an analytical balance.

The egg albumin used was a commercial product (Merck, impalpable powder); the plasma fractions were derived from citrated, freshly drawn, human blood. Lysed cell-free, blood fractions were prepared by alternately freezing and thawing defibrinated whole blood, centrifuging and collecting the supernatant fluid. These test samples were supplemented by dissolving the required amount of crystalline sugar in a given volume of the sample or by dissolving the solid material in the sugar solution.

RESULTS

The quantity of a meal ingested by individual mosquitoes which "fed" or "engorged", according to our method of classification, was determined. The average meal weights of the eight "fed" specimens was 0.58 mg. (range 0.17–0.80 mg.) and of the nine "engorged" females, 2.15 mg. (range 1.87–2.40 mg.). No overlapping of the values of these two groups was noted.

Marked differences were observed in the response of *A. aegypti* to the various

TABLE 1

The feeding preferences of Aedes aegypti *for various sugar solutions*

Concentration	Solution	Per Cent Total Mosquitoes		
		Unfed	Fed	Engorged
5%	Water	100	0	0
	Lactose	61	21	18
	Glucose	18	32	50
	Sucrose	*4*	*36*	*60*
	Glycerin	85	15	0
	Fructose	29	50	21
10%	Water	100	0	0
	Lactose	67	23	10
	Glucose	14	38	48
	Sucrose	*0*	*14*	*86*
	Glycerin	80	17	3
	Fructose	32	23	45

TABLE 2

The relative sweetness indexes for man and for certain Diptera

Solution	Sweetness Index			
	Man[1]	Phormia[2] (tarsi)	Calliphora[3] (tarsi)	Aedes aegypti[4]
Water	0	—	—	0
Lactose	16	0	0	12
Glucose	74	9	0.5	56
Sucrose	100	100	100	100
Glycerin	108	—	—	4
Fructose	173	132	18	52

[1] West and Todd, 1952a.
[2] Hassett, *et al.*, 1950.
[3] Haslinger, 1935.
[4] Present study, 10 per cent concentration.

preparations as determined by the extent of feeding (Table 1). At the 5 per cent concentration level, lactose, glycerin, and fructose were, like water, unattractive for engorgement, whereas glucose was found to be almost as effective as sucrose, the most attractive sugar. The differences were magnified, however, at the 10 per cent concentration level. Less than 10 per cent of the females engorged on water, lactose, or glycerin, 48 per cent engorged on glucose, and 45 per cent on fructose, whereas 86 per cent fed to repletion on the sucrose solution. Furthermore, all the mosquitoes that were offered sucrose fed to some degree. The preference for sucrose was demonstrable not only in terms of greater percentages of engorged females, but also in the time required to obtain engorged specimens. Those mosquitoes offered sucrose became engorged within a few minutes after the beginning of the feeding period, whereas much longer times were required by the mosquitoes given the other sugar solutions.

The relative sweetness scale for man is based on sucrose, which is arbitrarily assigned a value of 100. By transforming the above data and that obtained by others studying dipterous insects, it was possible to compare the sugar preferences of mosquitoes with those of other organisms (Table 2). This was based on the equivocal assumption that sweetness and preference are related for the mosquito. *Aedes aegypti* and the blue bottle fly (*Calliphora*) exhibited a much greater preference for sucrose than for fructose or glucose. Glycerin which has the same index for man as sucrose, was singularly unattractive to the mosquito. On the other hand, the black blowfly (*Phormia*) and man regarded fructose as the sweetest sugar followed by sucrose.

As a possible explanation of the response of mosquitoes to sucrose, the relative viscosities of the various solutions were determined. The values ranged from 1.26 to 1.33 and were not considered different; hence, it is unlikely that this physical property is related to sugar preference.

The affinity of mosquitoes for sucrose was demonstrated further by testing sucrose supplemented preparations (Table 3). It was observed that no engorgement was obtained with the aqueous or lactose supplemented solutions of egg

TABLE 3

The feeding preferences of Aedes aegypti *for sugar-supplemented 10% egg albumin solutions*

Solvent	Number of Mosquitoes	Per Cent Total Mosquitoes		
		Unfed	Fed	Engorged
Water	30	93	7	0
10% Lactose	22	100	0	0
10% Sucrose	19	11	16	73

albumin, whereas the addition of sucrose resulted in 73 per cent engorgement and 89 per cent total feeding. Similar results were obtained with plasma and acellular, lysed blood solutions. Egg deposition occurred in some cases following engorgement of these test fractions, but not after feeding on sucrose alone.

DISCUSSION

The amount of blood ingested by a mosquito has been shown to be related directly to the number of eggs deposited. However, meal intakes greater than about 1.75 mg./mosquito were excessive, because maximal egg production was attained at that level (Woke *et al.*, 1956). In the present study the average meal weight of "engorged" females was 2.15 mg. and thus in excess. These measurements were obtained using a simple classification method of the extent of feeding and a weighing technique which obviated the common practice of anesthetizing the insect (Roy, 1936; Woke, unpublished data). The adequacy of these intakes also has been demonstrated in other experiments, for maximal eggs/mosquito ratios were obtained using engorged females (Lang, 1956). These results provided valuable support for the validity of this classification method, which may be applicable in other fields of mosquito research involving the oral administration of chemical or infectious agents.

Sucrose alone or as a supplement to unattractive test samples induced engorgement of *A. aegypti*. An explanation for this preference may be an inherent or conditioned predilection for this disaccharide, for the plant saps and fruit juices, which form the natural diet of mosquitoes between blood meals, are especially rich in sucrose (West and Todd, 1952b).

Other investigators have shown that the destination of a blood meal in mosquitoes is "switched" or diverted from the midgut to the esophageal diverticula if sucrose is present (Day, 1954; Trembley, 1952). In our experience an average of 73 eggs/mosquito (5 tests) resulted after a sucrose supplemented blood meal as compared to 79 eggs/mosquito (3 tests) after a blood meal alone. Since this difference was not significant, these results indicated that the addition of sucrose does not alter the nutritional efficacy of the test preparations in respect to egg production.

SUMMARY

The preference indexes of several chemically defined sugar solutions were determined for adult female *Aedes aegypti*. These mosquitoes not only engorged on a solution of sucrose, the preferred sugar, but also fed to repletion on un-

attractive test preparations when supplemented with this disaccharide. This information was utilized in the development of a simple feeding procedure for this insect which would provide sizeable numbers of engorged mosquitoes.

REFERENCES

DAY, M. F., 1954. The mechanism of food distribution to midgut or diverticula in the mosquito, *Australian J. Biol. Sci.* **7:** 515–524.

DIMOND, J. B., LEA, A. O., BROOKS, R. F., AND DELONG, D. M., 1955. A preliminary note on some nutritional requirements for reproduction in female *Aedes aegypti*, *Ohio J. Sci.* **55:** 209–211.

GREENBERG, J., 1951. Some nutritional requirements of adult mosquitoes (*Aedes aegypti*) for oviposition, *J. Nutrition* **43:** 27–35.

HASLINGER, F., 1935. Über den Geschmacksinn von Calliphora erythrocephala Meigen und über die verwertung von Zuckern und Zuckeralcoholen durch diese Fliege, *Zeitschr. Vergleich. Physiol.* **22:** 614–640.

HASSETT, C. C., DETHIER, V. G., AND GANS, J., 1950. A comparison of nutritive values and taste thresholds of carbohydrates for the blowfly, *Biol. Bull.* **99:** 446–453.

KNIERIM, J. A., LEA, A. O., DIMOND, J. B., AND DELONG, D. M., 1955. Feeding adult mosquitoes on preserved blood to maintain egg production, *Mosquito News* **15:** 176–179.

LANG, CALVIN A., 1956. The influence of mating on egg production by *Aedes aegypti*, *Am. J. Trop. Med. & Hyg.* **5:** 909–914.

ROY, D. N., 1936. On the role of blood in ovulation in *Aedes aegypti* Linn., *Bull. Ent. Res.* **27:** 423–429.

TREMBLEY, HELEN L., 1952. The distribution of certain liquids in the esophageal diverticula and stomach of mosquitoes, *Am. J. Trop. Med. Hyg.* **1:** 693–710.

WALLIS, ROBERT C., 1954. The effect of population density and of NaCl concentrations in test series in laboratory experiments with ovipositing *Aedes aegypti*, *Mosquito News* **14:** 200–204.

WEST, E. S., AND TODD, W. R., 1952 a, *Textbook of Biochemistry*, Macmillan Company, 1st edit., p. 311.

WEST, E. S., AND TODD, W. R., 1952 b, *Textbook of Biochemistry*, Macmillan Company, 1st edit., p. 285.

WOKE, PAUL A., 1937. Effects of various blood fractions on egg production of *Aedes aegypti* Linn., *Amer. J. Hyg.* **25:** 372–380.

WOKE, PAUL A., ALLY, MONA S., AND ROSENBERGER, CHARLES R., JR., 1956. The numbers of eggs developed related to the quantities of blood ingested in *Aedes aegypti* (Linnaeus), *Ann. Ent. Soc. Amer.* (in press).

STREAM GRADIENT AS A CRITERION OF LOTIC HABITATS SUITABLE FOR *AUSTRALORBIS GLABRATUS* IN PUERTO RICO

HAROLD W. HARRY AND BILLY G. CUMBIE[1]

Tropical Research Medical Laboratory, U. S. Army, San Juan, Puerto Rico

In a previous paper the authors (1956) pointed out that *Australobris glabratus*, host of *Schistosoma mansoni* in Puerto Rico, occurs in both lotic and lenitic waters, but not in all of the subtypes within each class. Since all the evidence indicates that the former type of habitat is of particular importance in the epidemiology of schistosomiasis on the island, detailed studies have been made of the streams. In the previous paper it was pointed out that high gradient streams did not contain the snail, and that it was present in many, but not all, of the streams of lower gradient. The present account attempts to define the critical gradient in exact terms.

METHODS

The material presented is based on data from more than 350 stations in Puerto Rico and includes observations on biota, topography and water quality. These were correlated with data obtained from topographic maps. The gradient of the various reaches of each stream was determined by measuring the distance on the map between 10 meter contour intervals with dividers. The field observation stations, junctions with other important streams, changes in regional physiography, and the presence of major urbanizations were also noted. The nomenclature of streams used is that of the U. S. Army Map Service 1:25,000 map of Puerto Rico, E. 835, First Editions (AMS 1) 1948.

Choice of the appropriate reach of a stream for determining the critical gradient is largely arbitrary. Short adjacent reaches often have extremes of gradient value, particularly in headwater areas. Using an excessively long reach, e.g., the total length of a stream, is apt to destroy the usefulness of the criterion. Two streams of comparable length and fall may have entirely different gradient patterns. In the early stages of this study, after a correlation of tabulated gradients had been made with data on biota at various field stations, it was found that reaches of about 1,000 meters were the most practical for the determination of the critical gradient. This distance has been adopted as the standard unit in these studies.

The populations of *Australorbis* dealt with here have been separated into two categories, those associated with the main channel of the stream, and those in disjunct pools, marshy areas and smaller seepage tributaries. In nature the differentiation between the main stream and neighboring habitats is often difficult. Marginal habitats have been separated as "channel" and "disjunct"

[1] Field Team from Department of Medical Zoology, Walter Reed Army Institute of Research, Walter Reed Army Medical Center, Washington 12, D. C.

on the basis of how changes in the stream affected them. If changes in volume, temperature or chemical composition of the stream were distributed to the marginal habitats within a few minutes, they were considered a part of the main channel. Data were also obtained on the population of other snails, with special attention being given to those belonging to the closely related genus *Tropicorbis*. Twelve streams that were studied have been selected to present some of the observations made.

RESULTS

Sabana Llana streams. The profiles of three streams of the Sabana Llana type are shown in Figure 1. All of these streams are on alluvium for most of their extent but above this they are spring-fed, cutting through thick-bedded, well indurated Cretaceous shales, which are beveled by the Caguana Peneplane. The gradient values are low even in the permanent portions of the headwaters. The Dona Ana enters the Rio Piedras a few hundred meters above the tidal zone of the latter. The other two streams flow directly into brackish bays in the vicinity of San Juan.

At station 148, on the Dona Ana, a population of *Australorbis* was present during the observation period of about one year, in spite of changes produced by a new housing development. Infected snails were frequently found in the collections from this station. The snail was not found at stations 131 and 129, even though it is known to occur farther upstream. This irregular distribution is common in some low gradient streams, particularly in the Sabana Llana series. At station 131 a dense population of the snail was found in a disjunct ox-bow produced by straightening of the stream. *Tropicorbis decipiens* occurs in

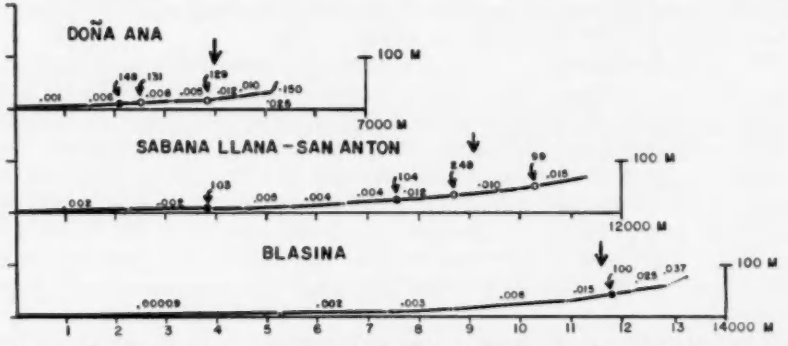


FIG. 1. Gradient patterns of streams of the Sabana Llana type. The scale is in meters, the vertical scale represents the distance above sea level. Gradient values for each reach of the stream are written above the profile. Open circles indicate collecting stations at which *A. glabratus* is not established. Closed circles indicate stations where the snail has been found. The station numbers are written near each circle. The vertical arrows indicate the upstream extent of alluvium. Two such arrows along a stream represent an alluviated valley, with no alluvium along the stream below the downstream arrow.

temporary puddles in a dirt road at this point, but it is only rarely found in the main channel a few meters away.

A population of *Australorbis* was present at station 100 on the Blasina and *S. mansoni* was found in several collections. This station is of interest because it is so far upstream. Snails are known to be present at various points below this station. The stream is only one to three meters wide at the station but is bounded by about an acre of spring-fed, caladium-filled marsh. The snail is also present in the marsh, which undoubtedly serves as a reservoir that assists in maintaining a population in the main channel.

While *Australorbis* has not been found at two arbitrarily selected upstream stations on the Sabana Llana-San Anton (99 and 248), marshy, spring-fed sections similar to those of the upper Blasina are known in the headwater areas. At station 104 a variety of freshwater snails occur. Besides *Australorbis* there are three other species of Planorbidae; one species each of Physidae, Lymnaeidae, Amnicolidae and Ancylidae; and two species of Ampulariidae. The last two species seem to have been recently introduced into Puerto Rico, perhaps by tropical aquarium fish fanciers. One of these species *Ceratodes cornuarietis* (*Marisa* of authors) has been reported to eliminate *Australorbis* populations under certain conditions (Oliver-González *et al.*, 1956, Chernin *et al.*, 1956). Our observations on the Sabana Llana indicate that *Australorbis* may disappear where *Ceratodes* is able to produce large populations, but with both snails the

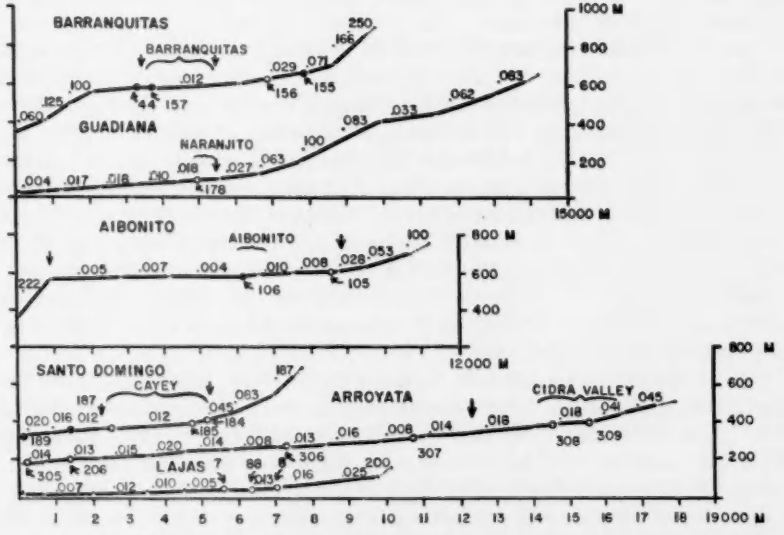


FIG. 2. Selected gradient patterns of streams in the Rio de la Plata drainage system. The presence of a town along a stream is shown by a horizontal bracket. See text for an explanation of the Cidra Valley. Other symbols as in Figure 1.

density is quite variable. At station 103 moderate populations of *Ceratodes* and *Australorbis* coexisted over a two year period.

Rio de la Plata watershed. Figure 2 illustrates the variety of stream gradient patterns that occur in the Rio de la Plata drainage system. With two exceptions all the streams shown flow directly into the Plata. The Rio Aibonita flows into the Rio Usabon a short distance above the junction of the latter with the Quebrada Barranquitas, and the Usabon then enters the main channel of the Plata about halfway between the Quebrada Santo Domingo and the Rio Arroyata. In the Cayey, Aibonito and Cidra Valleys, there are other streams in the headwaters of the Plata that have not been considered in this paper. These have populations of *Australorbis* and may contribute to the incidence of schistosomiasis in these areas.

The Rio Lajas is a western tributary of the Plata. It flows entirely on Tertiary Limestone and lacks an alluvial margin. It is in a broad, U-shaped valley of gently sloping walls topped by pepino hills of karst topography. Several small, spring-fed tributaries flow into it. There are a few houses in the valley but no urbanization. Sewage pollution, therefore, is a negligible factor in the Lajas. Although its general gradient is not as low as the streams in the Sabana Llana type, it is well below the value thought to be critical (see below). *T. decipiens* occurs in temporary puddles near station 88 but *Australorbis* has not been found. In this case it is believed that water quality is the factor that excludes the vector snail. As reported in the previous paper, *Australorbis* has not been found in streams of the limestone area.

The other streams shown in Figure 2 are derived from mixed volcanic rocks of the Oldland Province. The Rio Arroyata is an eastern tributary and arises in the Cidra Valley, which is a Caguana Peneplane surface. Most of this valley is in the headwaters of the Rio Bayamon, an independent drainage system. The town of Cidra is located on the low drainage divide between the two rivers. Major urbanization does not occur adjacent to the permanent reaches of either stream. If pollution from Cidra enters the Arroyata, the effect is not reflected in its biota. There are no marked changes in the gradient between the Cidra Valley and the upstream extension of the alluvium, which forms a narrow margin along most of its lower reach. In spite of an alluvial margin and a gradient below that believed to be critical, no *Australorbis* have been found at six stations. Small populations of *T. albicans* occur at and below station 306.

The Rio Guadiana is a western tributary of the Plata, entering between the two streams given above. Above the town of Naranjito the gradient is believed to be too steep for *Australorbis*. At the lower edge of the town, where the gradient of the reach is 0.018 and there is slight evidence of pollution biota in marginal pools, no *Australorbis* have been found (Harry and Cumbie, 1956. Figure 5). The snail was not found in a cursory examination of a standard reach above station 178 and most of the stream below it. Additional evidence that the snail is not present in the stream has been indicated by Weller and Dammin (1945). They found only one case of schistosomiasis in 70 individuals examined from the Naranjito Municipality.

At two upstream stations on the Barranquitas there is no gross evidence of pollution, the gradient is high, the biota depauperate, and *Australorbis* has not been found. In the vicinity of the town of Barranquitas two loops of the stream bound crescents of alluvium. At times of low water the biota at stations 157 and 44 indicates mild pollution. Snail populations are generally low at both of these stations, less than one per linear meter of shore line, but the incidence of *S. mansoni* in the colony sometimes exceeds 50 per cent. The town has remained a persistent focus for schistosomiasis since it was first surveyed by Hoffman and Faust (1934). The gradient of this reach of the stream is less than that considered to be critical but it is just below higher gradient headwater reaches, and after heavy showers there is considerable flushing. At times, therefore, no snails can be found. Judging from the biotic manifestations of pollution, there is much more organic enrichment relative to the size of the stream at station 157 and slightly above, than there is entering the Guadiana at Naranjito. In view of the gradient values along the Barranquitas, and the periodic flushing of snails, it is possible that the vector could be excluded from the stream if pollution was stopped.

The Rio Aibonito is similar to the Barranquitas, but more of it is low gradient and bordered by alluvium. The station below the upper boundary of the alluvium (105), near the beginning of the reaches which are believed to have a suitable gradient, is negative for *Australorbis*. Station 106 is located within the boundaries of the town of Aibonito and there is biotic evidence of mild pollution. Infected snails were reported from the Aibonito Valley by Hoffman and Faust (1934) but none were found in the single small collection made at station 106.

As it emerges from the hills (station 184) the Quebrada Santo Domingo at Cayey (Figure 4) has extensive slum areas along its banks to the point where it leaves the upland alluviated valley (station 186). Scattered clusters of homes of the same type are found downstream as far as station 187. Below station 186 the stream flows through a narrow zone of foothills before joining the Plata. Pollution biota increases gradually downstream from station 184. At station 186 pollution is intense, with no dissolved oxygen, and an abundance of *Sphaerotilus*. Station 187 is a zone of moderate recovery and at station 189 there is normally little evidence of pollution but it has a dense population of organisms commonly found in nonpolluted waters. *Australorbis* is found occasionally at station 187 but at 189 it occurs by the hundreds within a linear meter of stream. The segment of the stream in the region of station 189 is slightly shorter than the standard reach. Its gradient of 0.020 represents the maximum in which we have found *Australorbis* in Puerto Rico. In the absence of organic enrichment, this station would probably be less favorable for maintaining a snail population. *T. albicans* is found in small numbers at stations 184, 187 and 189.

Miscellaneous streams. Streams from three independent drainage systems are shown in Figure 3. The Rio Orocovis is a tributary of the Rio Manati, which it joins at 200 meters' elevation. The alluvial margin of the Manati drainage system extends well upstream along the Orocovis. The steep gradient values of the

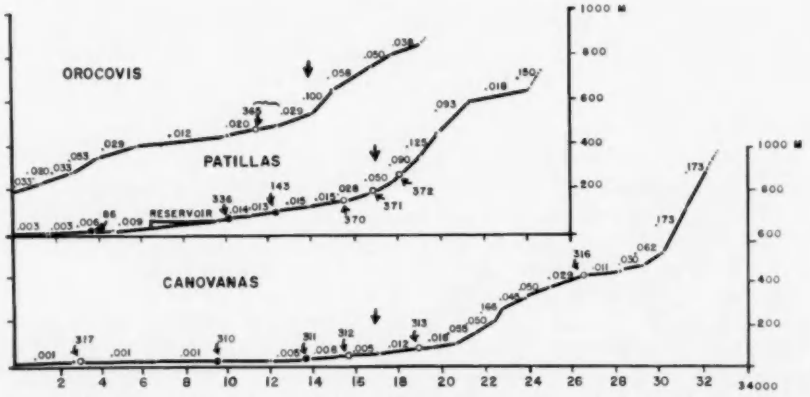


FIG. 3. Gradient patterns of three independent drainage systems. Symbols as in Figure 1.

stream, in the section bordered by alluvium, is typical of many Puerto Rican streams mentioned in the previous paper. They have undergone entrenchment and are again cutting bedrock. At the single station on this stream (365), it is 10 to 20 meters wide and there is no gross biotic evidence of pollution from the town. *Australorbis* was not found and surveys of similar streams indicate that they are not suitable habitats for this snail. Weller and Dammin (1945) found only one positive stool in 145 examined from the municipality of Orocovis, indicating that transmission was not taking place in the area.

The Rio Canovanas is one of the major streams that flows from the northern flank of el Yunque. It joins the Rio Grande de Loiza just above the tidal reaches of the latter. The steep gradient of the upper third of the stream is broken slightly by a small plateau (station 316). This appears to be a remnant of the Caguana Peneplane. About half of the stream is on alluvium but it has no major urbanizations. *Australorbis* is not present on the plateau, or in the first two stations at the lower level. At station 313, however, a few snails were found in a disjunct pool formed by seepage from an alluvial terrace. At stations 311 and 310 *Australorbis* populations are extremely sparse and the snail is not present at station 317, near the mouth of the stream. None of the snails collected in this stream were parasitized. It is difficult to determine from the available literature what the incidence of schistosomiasis is in the human population along this stream. Weller and Dammin (1945) reported an incidence of 6.8 per cent in the municipio of Loiza, but the Canovanas is not the only low gradient, alluvial stream draining the area.

The Rio Patillas originates on the southern flank of the Cordillera Central and extends out on an alluvium. The latter is quite arid and a dam has been built in the lower third of the stream. The beds on the alluvium below the dam would undoubtedly be dry throughout most of the year without the diversion of water from an aqueduct a short distance above station 86. *T. riisei* is present at this station and occasionally there are a few *Australorbis*. At station 143,

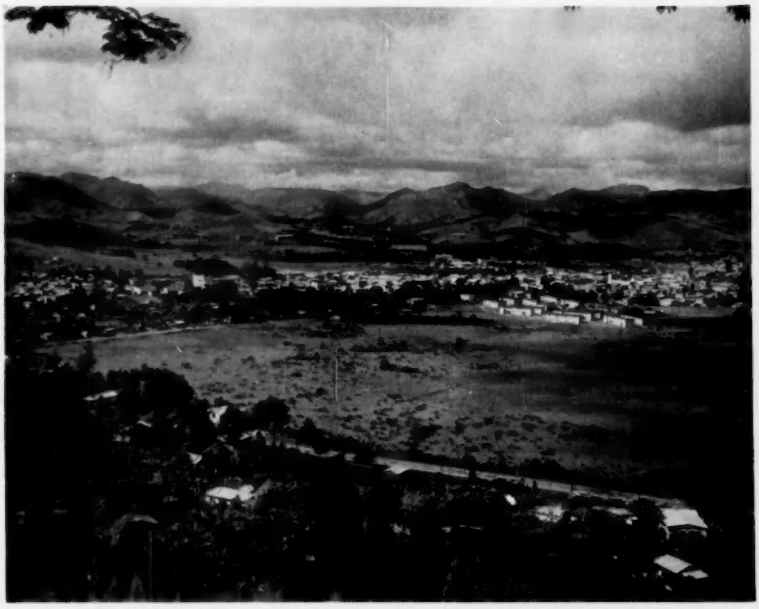


FIG. 4. The upland alluviated valley at Cayey. The Quebrada Santo Domingo flows from left to right within the urbanized area. Station 184 is at extreme left, and 186 just beyond the extreme right margin of the picture. The Saint John Peneplane forms the skyline, and the low foothills may represent the Caguana Peneplane.

populations of this snail are moderate to abundant and infected snails have been found but the incidence is low. Only occasional specimens of *Australorbis* were found at station 336. Above these stations *Australorbis* was not present at two additional sites on the alluvium or at one in the Oldland Province. *Australorbis* has not been found in the reservoir but a sparse population of *T. riisei* occurs there.

DISCUSSION

The low gradient headwater areas of streams of the Sabana Llana series (Figure 1) and the Arroyata River (Figure 2) of the Cidra Valley are typical of streams in areas where alluvium is absent and the Caguana Peneplane is the dominating surface. Lower gradients are encountered along some streams margined by alluvium, as exemplified by the lower reaches of the streams of the Sabana Llana series. Long reaches of streams with a gradient of less than 0.010 are often favorable for *Australorbis*. Low gradient reaches immediately downstream from extensive high gradient headwater areas generally are unfavorable for *Australorbis*. *Tropicorbis riisei* and *T. albicans*, two planorbids sometimes confused with *A. glabratus*, may occur farther upstream than *Australorbis*, but not necessarily in reaches of steeper gradient. *Australorbis* may occur in dis-

junct marginal pools upstream from where it is found established as a main channel population (station 313 on the Rio Canovanas, Figure 3). Snails may be found occasionally in a reach which is normally too steep for the development of a population, when they are established upstream. Their presence is due to flushing and they remain in the steeper reaches for relatively short periods.

While the gradient does have an effect on the distribution of the schistosome vector, it is obvious from the data presented above that other factors also play a role. It is apparent that intense pollution produces adverse conditions but that a certain amount of organic enrichment is advantageous to the snail. As has been pointed out, in certain places it appears that *Australorbis* populations could be eradicated or greatly reduced if pollution was eliminated. Even if this were not the case it would, presumably lower the incidence of the infection in the snails. *Australorbis* does not seem to live in streams that come from limestone and some other formations. There is also evidence that critical levels of dissolved solids are a factor in the distribution of this snail. Studies on the chemical composition of freshwater habitats in Puerto Rico will be the subject of another paper in this series.

SUMMARY

In the examination of more than 350 collecting stations it was observed that populations of *Australorbis glabratus*, the vector for *Schistosoma mansoni* in Puerto Rico, are not maintained in reaches of streams steeper than 20 meters fall per 1000 meters of length. Water quality and other factors apparently cause the spotty distribution observed in reaches with suitable gradients.

ACKNOWLEDGMENTS

We wish to express our gratitude to Dr. Donald B. McMullen for his encouragement during the study, and to Mr. C. Levitt Smith, Jr. for technical assistance. The review of the manuscript by Lt. Colonel Elmer Chaffee is much appreciated.

LITERATURE CITED

HARRY, H. W., AND CUMBIE, B. G., 1956. The relation of physiography to the types of freshwater environments and the presence of *Australorbis glabratus* (Say) in Puerto Rico, *Am. J. Trop. Med. & Hyg.* To be published.

CHERNIN, E., MICHELSON, E. H., AND AUGUSTINE, D. L., 1956. Studies on the biological control of schistosome-bearing snails. I. The control of *Australorbis glabratus* population by the snail, *Marisa cornuarietis*, under laboratory conditions, *Am. J. Trop. Med. & Hyg.* **5**: 297–307.

HOFFMAN, W. A., AND FAUST, E. C., 1934. Studies on schistosomiasis in Puerto Rico. II. The epidemiology and geographical distribution of schistosomiasis mansoni in Puerto Rico. I. Epidemiology of the infection on the island, *Puerto Rico Jour. Pub. Health and Trop. Med.* **9**. 228–254.

OLIVER-GONZÁLEZ, J., BAUMAN, P. M., AND BENENSEN, A. S., 1956. Effect of the snail *Marisa cornuarietis* on *Australorbis glabratus* in natural bodies of water in Puerto Rico, *Amer. J. Trop. Med. and Hyg.* **5**: 290–296.

WELLER, T. H., AND DAMMIN, G. J., 1945. The incidence and distribution of *Schistosoma mansoni* and other helminths in Puerto Rico, *Puerto Rico Jour. Pub. Health and Trop. Med.* **21**: 125–147.

REPORT OF A CASE OF HUMAN INTESTINAL MYIASIS CAUSED BY *LEPTOCERA VENALICIA*

DON W. MICKS AND J. WARREN McKIBBEN

Laboratory of Medical Entomology, Department of Preventive Medicine and Public Health and Department of Internal Medicine, University of Texas Medical Branch, Galveston

More than twenty-five species of flies representing at least thirteen different families of Diptera have been implicated in enteric myiasis. Patton (1921, 1922) classifies most fly larvae which may invade the gastrointestinal tract as accidental myiasis-producing Diptera inasmuch as these species may breed in food and therefore be accidentally ingested. As pointed out by James (1947), many cases reported to be enteric myiasis may be attributed to contamination of stools or similar misleading circumstances. On the other hand, it seems likely that a considerable number of cases of true intestinal myiasis may be completely overlooked or unreported.

The purpose of this paper is to report a case of human intestinal myiasis due to *Leptocera venalicia*, family Sphaeroceridae, a fly not previously reported to be involved in any type of human myiasis.

CASE REPORT

The patient (C. L. S.) was a 46-year-old white meter-repairman, who reported to the clinic on February 5, 1952, carrying a small glass jar containing many fly larvae, which he had collected from his stool specimens. He had first noticed the presence of these larvae in his stools five weeks previously and had been finding them at irregular intervals since. He remarked that "these white worms will spring a distance of three to four inches."

The patient had experienced gastric distress and flatulence since a spinal operation seven months previously. He stated that as long as the larvae appeared in his stools he also had lower abdominal cramping pain, increased flatulence, a mild flare-up of an old arthritis involving his knees, ankles and neck, a slight increase in thirst, and tendency toward insomnia. He had been receiving an antihistamine for four weeks (50 mgm. three to four times a day) prior to his first visit to the clinic. He noted some reduction in epigastric distress, but no change in the number or activity of larvae in his stools. He gave no history of coughing up larvae or blowing them out of his nose.

The patient had worked as a meter-repairman for the past ten years. He prepared and took his lunch to work every day. This usually consisted of cheese, lunch meat, or boiled ham sandwiches, and fresh fruit. The food was purchased in a clean, modern store and said to be kept in a refrigerator until used. Breakfast and supper were usually heated meals. The patient had four children, but none of them nor his wife had seen larvae in their stools.

On physical examination by one of us (JWM), his blood pressure was 130/88. The pulse rate was 80 per minute, the respiration rate 20 per minute and his temperature was 98°F. The patient was a heavy set, white male, who did not

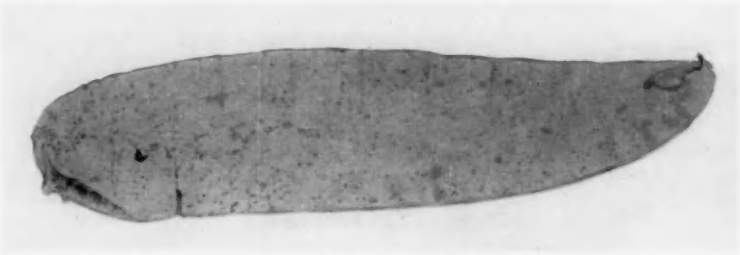


FIG. 1. Mature larva of *L. venalicia* (19×)

appear ill. The physical examination was essentially negative as were the laboratory findings. A barium enema revealed a normal colon. Subsequently, a gastric analysis revealed a low HCl content. At this time living larvae were sent to one of us (DWM) for identification. Some of the larvae along with adult flies reared therefrom were sent to Dr. Maurice T. James for identification. He identified them as *Leptocera venalicia* (OS.), family Sphaeroceridae (= Borboridae). No treatment was given at this time. When the patient returned on February 18, 1952, to obtain the results of his laboratory tests, he reported that he was still passing larvae in his stools. They would appear in considerable numbers after a cathartic, but if he watched a normal stool, a few larvae could eventually be seen.

A month later the patient returned to the clinic and reported that a local physician had given him two courses of "crystoids" one week apart starting February 21. The treatment had consisted of a light evening meal followed by five "crystoids" the next morning, and a saline cathartic in twenty-four hours. No more larvae were seen although the patient had been looking for them. At this time he brought a bottle containing dead flies caught in his bedroom and a stool specimen which was negative for fly larvae. The patient was last seen in the clinic on May 12, 1952, at which time he reported the reappearance of larvae in his stools for at least two days. Larvae brought in at this time were identified as *L. venalicia* (Fig. 1.). A complete blood count showed a 9 per cent eosinophilia.

A visit was made to the patient's home in October, 1952. It was located some fifteen miles east of Bay City, Texas, in isolated country. The house was unpainted, and flies had free access to the interior through holes in the walls and kitchen floor although there was screening on the doors and windows. There were cats, ducks, chickens and emaciated dogs living under the kitchen floor. A combination corn mill, chicken house, hog pen, cow barn and machinery storage stood twenty feet from the house in one direction and an ancient latrine which had obviously been in use for a good many years was nearby. Garbage was collected outside the porch in an open receptacle and the cans dumped about forty feet away, the rest being fed to the hogs. The entire area had a heavy fly population and optimum conditions for myiasis prevailed.

DISCUSSION

Cases of intestinal myiasis may vary in severity according to the numbers of larvae involved, their ability to survive in the intestine, and the general condi-

tion of the patient. The significance of this latter factor is borne out by the experimental work of Kenny (1945) who found marked variation in the response of sixty human volunteers each of whom was fed twenty living fly larvae in gelatin capsules. He was able to divide the patients into five separate groups according to the symptoms. Ten of his cases were completely asymptomatic. This evidence lends support to the belief that many individuals probably pass larvae in their stools with no evidence of intestinal disturbance. It likewise seems probable that in cases of myiasis where abdominal cramps and diarrhea are the prominent symptoms and the stool has not been examined for larvae, the individual may assume he has bacterial food poisoning. In the present case the symptoms were not particularly severe nor did they appear to vary appreciably with the numbers of immature stages present in the stool. Blood was not visible in any of the patient's stool specimens and a proctoscopic examination was completely negative, revealing normal-appearing mucosa.

Hundreds of larvae and pupae of *L. venalicia* were present in the patient's stools although relatively few were found in hard stools. The fact that pupae were also found indicates that the larvae were not only able to survive in the intestine, but were also able to undergo partial development therein. In this connection, it is interesting to note that practically all larvae which were placed in 70 per cent alcohol continued development to the pupal stage. The larvae had the peculiar habit of bringing their posterior and anterior ends together and springing several inches. This habit, which is also characteristic of the cheese-skipper larvae, is not especially surprising inasmuch as the Sphaeroceridae are fairly closely related to the Piophilidae. The larvae of *L. venalicia* measure approximately 5 mm. in length and are extremely active. Perhaps their most striking character is the form of the prothoracic spiracles. These carry over into the anterior horn-like projections of the pupae. Both larvae and pupae were recovered from freshly passed stools; however, adult flies were not found. *L. venalicia* is an old world tropical or subtropical species which was supposedly introduced into the southeastern United States through the slave trade of the early days. Very little is known about its biology or immature forms (James, personal communication.).

SUMMARY

A case of human intestinal myiasis due to *Leptocera venalicia* is reported. This species apparently has not been previously reported in connection with human myiasis. Larvae and pupae appeared in various stool specimens over a period of five months. Except for occasional episodes of gastric distress and abdominal cramps, the patient was essentially asymptomatic. The patient probably ingested eggs or larvae with contaminated meat or cheese. The heavy fly population at the patient's home was conducive to myiasis.

ACKNOWLEDGEMENT

Sincere appreciation is extended to Dr. Maurice T. James for his kind assistance in making identifications and for supplying other information about the species involved.

REFERENCES

JAMES, M. T., 1947. The flies that cause myiasis in man, *USDA Misc. Pub.* No. 631, Washington, D. C. 175 pp.

KENNEY, M., 1945. Experimental intestinal myiasis in man, *Proc. Soc. Exp. Biol. & Med.* **60:** 235–237.

PATTON, W. S., 1921. Notes on the myiasis-producing Diptera of man and animals, *Bul. Ent. Res.* **12:** 239–261.

PATTON, W. S., 1922. Some notes on Indian Calliphorinae. VII. Additional cases of myiasis caused by the larvae of *Chrysomyia bezziana* Vill., together with some notes on the Diptera which cause myiasis in man and animals, *Indian Jour. Med. Res.* **9:** 654–682.

ERRATUM

In the article by T. T. Mackie, J. W. Mackie, C. M. Vaughn, N. N. Gleason, B. G. Greenberg, E. S. Nenninger, M. N. Lunde, L. L. A. Moore, J. A. Kluttz and M. O. Taliafero entitled "Intestinal Parasitic Infections in Forsyth County, North Carolina. V. Prevalences of Individual Parasites" and published in the January, 1956 issue of this Journal (vol. 5, p. 50) the formula four lines from the bottom on page 50 should read as follows:

$$\left\{\begin{array}{l}\text{Percentage of school children who were positive}\end{array}\right\} \times \left\{\begin{array}{l}\text{Percentage of positive family members of positive school children}\end{array}\right\} + \left\{\begin{array}{l}\text{Percentage of school children who were negative}\end{array}\right\} \times \left\{\begin{array}{l}\text{Percentage of positive family members of negative school children}\end{array}\right\}$$

BOOKS RECEIVED

Composting, by Harold B. Gotaas. 205 pages illustrated. World Health Organization. New York, distributed by Columbia University Press, 1956. Cloth $5.00.

Diseases of the Skin, by Richard L. Sutton. 1972 pp. illustrated. St. Louis, C. V. Mosby Company, 1956. $29.50.

Jahreszeit und Witterung im Seuchengeschehen, by Wilhelm Donle. 191 pp. illustrated. Stuttgart, Ferdinand Enke Verlag, 1956. Geheftet: DM 25; Gansleinen: DM 27.20.

Cryptococcosis, Torulosis or European Blastomycosis, by M. L. Littman and Lorenz E. Zimmerman. 205 pp. illustrated. New York, Grune & Stratton, Inc., 1956. $8.50.

Annual Epidemiological and Vital Statistics, 1953, World Health Organization. 571 pages. New York, distributed by Columbia University Press, 1956. $10.00.

Medical Research: A Midcentury Survey, published for The American Foundation. 1505 pages. Boston, Little, Brown and Company, 1955. $15.00.

The History of the School of Tropical Medicine in London, by Sir Philip Manson-Bahr. 329 pp. illustrated. London: H. K. Lewis & Co., Ltd., 1956. £2. 10s. 0d.

The Training of Sanitary Engineers, School and Programmes in Europe and in the United States, by Milivoj Petrik. World Health Organization Monograph Series No. 32. 151 pp. New York, distributed by Columbia University Press, 1956. Cloth $4.00.

BOOK REVIEWS

Veterinary Parasitology, by Geoffrey Lapage, M.D., M.Sc., M.A., M. Inst. Biol. 964 pp., 34 plates. Springfield, Illinois: Charles C Thomas, 1956. Price $12.75.

According to the author, he has attempted to provide an account of the anatomy, biology, pathology, epidemiology and control of the parasites that affect the health of domesticated animals, and according to the reviewer he has succeeded well. This book is an amplification of one aspect of parasitism discussed in general in the author's well-known monograph *Parasitic Animals* (1951). The parasitism of helminths, arthropods and protozoa at the expense of British farm stock is thoroughly covered. The veterinarian, the farmer, the livestock disease control agency, the teacher and student of biology, comparative pathology, epidemiology and preventive medicine can find here a precise and informative description of the parasite in its zoological setting. It recommends itself both as a text and as a reference book.

The illustrative material in this volume is spectacular. The drawings, of which there are almost 500, have set a standard in their illustrative quality. In a simple manner they reveal the characteristic features of the parasites. This information, along with the text, is then summarized in photographic plates which show the parasite alone and in host tissue and the grossly visible changes suffered by the host.

Two introductory chapters concerning the general aspects of parasitism prepare the reader with the organization and principles, the framework, for the details that follow. The remainder of the book is taken up with an orderly presentation according to the biological classification of the parasites. The parasites themselves, their relationship with their hosts, the hosts' reactions and the methods of control are described masterfully. The description of these associations is excellently balanced, so that despite their variety and complexity they can be seen as a whole. The precise terminology is given, but does not become ponderous. The author has made every effort to make a vast amount of material comprehensible to the greatest possible number of readers.

The best in the art of printing and binding has been used in the preparation of this book.

K. F. Meyer

Annual Review of Entomology, Volume 1, 1956, edited by E. A. STEINHAUS, R. F. SMITH *et al.* 466 pp. illus. Stanford, California: Annual Reviews, Inc. in cooperation with Entomological Society of America, 1956. $7.00.

This volume represents a new venture of the Entomological Society of America. The purpose is for leading workers in each special area of entomology to provide objective summary reviews and appraisals of the current status of knowledge in their selected active fields of research. The 21 papers making up the volume will not be reviewed in detail. Papers of particular interest to readers of this journal are on insect nutrition, arthropod resistance to chemicals, the mode of action of insecticides, repellents, non-biting flies and disease, and veterinary and medical acarology.

As would be expected with 27 authors contributing 21 review articles, there is considerable variation in presentation and approach. However, in each contribution it is obvious that an exhaustive review and interpretation of the literature has been made, and the bibliographies are extensive. In most instances the authors felt it was essential to review the historical background of their subject area. It is anticipated that future reviews of the same subjects will be limited to a current period. Each author has done an outstanding service by his contribution. It is indicated that Volume 2 is in preparation and is made up of 22 review papers, of which all but two will be on new subject areas not covered in Volume 1.

The Society, editors and authors are to be commended for undertaking this project. The first and the successive volumes will provide a most valued summary for all workers in entomology and a ready reference and review source for other professional workers who must keep abreast of developments in entomology.

W. C. REEVES

Laboratory Identification of Pathogenic Fungi Simplified, by ELIZABETH L. HAZEN, PH.D., Associate Bacteriologist (Mycology) and FRANK CURTIS REED, Laboratory Illustrator and Photographer, Division of Laboratories and Research, New York State Department of Health, Albany, New York. 108 pp., Charles C. Thomas, Springfield, Ill. 1955 $5.50

This monograph is one of a series designated by the publisher as "American Lectures in Tests and Techniques". For the laboratory worker who is confronted with the ofttimes confusing and troublesome isolation of fungi from clinical specimens and their ultimate identification this small handbook should indeed prove a worthy companion. A technician who is not familiar with mycologic techniques or who does not have a knowledge of the structural features of the pathogenic fungi would find this text of limited aid and would have to turn to other sources for the required basic information.

Only those mycoses are considered which are commonly encountered in North America. There are two sections in the monograph; the first concerned with the superficial mycoses and the second with the deep-seated mycoses. The presentation of material for each of the fungus infections follows the same pattern throughout the text. There is first a brief consideration of the genus followed by a more detailed description of the important species within the genus. In the section dealing with the dermatophytes which covers approximately one half of the book each species is briefly considered as the etiologic agent of the characteristic infection with which it is associated. This is followed by a description of its cultural and microscopic characteristics. In every instance throughout the monograph these concise descriptions are limited to one page. Media recommended for the favoring of the development of both macroscopic and microscopic differential features of the fungus along with a description of these features are delineated in two rectangular boxes on the opposite page. Thus one obtains within the compass of two apposing pages a sort of birds-eye view of the etiology and differential mycologic diagnosis. These pages in turn are followed by photomicrographs of culture mounts and photographs of colonies.

The textual arrangement of the section devoted to the deep-seated mycoses is essen-

tially that of the previous section. The types of specimens suitable for examination and laboratory animals susceptible to infection are noted. Nocardiosis is limited to a consideration of *Nocardia asteroides* and maduromycosis to *Monosporum apiospermum*. In the section dealing with moniliasis only *Candida albicans* is considered. Perhaps rightly so but a beginner may be led astray when there is no mention of the other *Candida* species so frequently isolated from clinical materials. The differential diagnostic advantage of corn meal agar for the production of chlamydospores should have received more emphasis in view of the pitfalls encountered in separating *C. albicans* from the other species.

The particular feature of the monograph which will appeal to a great many readers is the large number of photographs throughout the text which illustrate in a most effective manner the macroscopic and microscopic features of each of the species of pathogenic fungi considered. No line drawings are used but they are not necessary when such clearly defined photomicrographs are available for guidance. Formulae of media noted in the text as well as a list of pertinent references are given. The book is well made, the large type print is exceptionally clear and the paper of excellent quality. The objective of the monograph as indicated in the title has indeed been effectively accomplished in this slim informative book which one keeps close at hand on the laboratory shelf for repeated consultation.

HERBERT G. JOHNSTONE

Hastening the decline of malaria

'DARAPRIM'® brand

Pyrimethamine

"... the most efficient suppressive agent known, ..."

"... of extraordinary effectiveness against erythrocytic parasites of all the malarias."

"... probably has some action against the primary exo-erythrocytic forms of all the malarias, ..."

"... is active against the late tissue forms of *Plasmodium vivax* ..."

"... exhibits a phenomenal effect on gametocytes. ... the parasites are not able to develop beyond the early oöcyst stage, thereby blocking the chain of transmission."

Coatney, G. R. : Industry and Tropical Health, Volume II, 1955.

For the treatment of an acute attack 'Daraprim' should be supplemented with a fast-acting schizontocide.

safe • *economical* • *tasteless*

Full information will be sent on request.

Tablet of 25 mg.
Supplied in boxes of 30 and bottles of 1,000.

BURROUGHS WELLCOME & CO. (U.S.A.) INC., Tuckahoe, New York

New DIFCO *Antisera*

BACTO-*C* PROTEIN ANTISERUM

for detection and quantitation of inflammatory diseases.

*

BACTO-GROUP *A* STREPTOCOCCAL ANTISERUM

for detection of Group A Streptococci

*

BACTO-*E. COLI OB* AND *O* ANTISERA

for identification of Enteropathogenic E. Coli Serotypes

Specify DIFCO *Antisera*

POTENT • SPECIFIC • RELIABLE

AVAILABLE ON REQUEST

Booklet 153—Bacto-*C* Protein Antiserum

Booklet 154—Bacto-*E. Coli OB* and *O* Antisera

Booklet 155—Bacto-Group *A* Streptococcal Antiserum

DIFCO LABORATORIES

DETROIT 1, MICHIGAN